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Factors Controlling Nickel Bioavailability and Effects On Benthic Invertebrates in Hardwater Freshwater Streams

Kevin Wayne Custer
Wright State University

FACTORS CONTROLLING NICKEL BIOAVAILABILITY AND EFFECTS ON BENTHIC INVERTEBRATES IN HARDWATER FRESHWATER STREAMS

A dissertation submitted in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy

By

KEVIN WAYNE CUSTER
M.S. Wright State University
B.S. The Ohio State University
A.S. Sinclair Community College

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I HEREBY RECOMMEND THAT THE DISSERTATION PEREPARED UNDER MY SUPERVISION BY Kevin Wayne Custer ENTITLED Factors Controlling Nickel Bioavailability and Effects on Benthic Invertebrates in Hardwater Freshwater Streams BE ACCEPTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS OF THE DEGREE OF Doctor of Philosophy

G. Allen Burton, Jr. Ph.D
Dissertation Director

Donald Cipollini, Ph.D
Program Director

Andrew Hsu, Ph.D.
Dean, Graduate School

Committee on
Final Examination

G. Allen Burton, Jr., Ph.D

William H. Clements, Ph.D

Joseph M. Culp, Ph.D

David F. Dominic, Ph.D

Chad R. Hammerschmidt, Ph.D

ABSTRACT

Custer, Kevin Wayne, Ph.D., Environmental Sciences Ph.D Program, Wright State University, 2012. Factors Controlling Nickel Bioavailability and Effects on Benthic Invertebrates in Hardwater Freshwater Streams.

Sediments in aquatic ecosystems function ecologically as habitat, food, and refugia that aid in reproduction processes, and chemically as sources and sinks for contaminants. Sediment contamination from metals and organics has been linked to numerous health and ecological effects, extending from fish consumption advisories to endangered species listings. This dissertation research examines Ni bioavailability (simultaneously extracted metal (SEM)/acid volatile sulfide (AVS) models) and toxicity in five separate studies using Ni-spiked sediments in a variety of designs, and mainly with two different sediment types (low AVS, total organic carbon (TOC), and high AVS, TOC).

Two separate streamside mesocosm Ni experiments indicated that benthic communities (Ephemeroptera, abundance, taxa richness) responded negatively to increasing SEM_{Ni}/AVS and (SEM_{Ni}-AVS)/*foc* models (increasing Ni bioavailability), and these communities demonstrated a sediment type preference. During a Ni-sediment flow-thru study, Ni toxicity was tested on four indigenous aquatic insects (*Anthopotamus verticis*, *Stenonema spp*., *Isonychia spp*., and *Psephenus herricki*) and two surrogate organisms (*Hyalella azteca* and *Chironomus dilutus*). Of the indigenous insects, *A. verticis* and *Stenonema spp.* were the most sensitive to Ni-spiked sediments in this study.

Overall, *H. azteca* was the most sensitive to total Ni, and *A. verticis* was most sensitive to the bioavailable fraction (SEM_{Ni}/AVS). Ni toxicity then examined natural benthic macroinvertebrate community colonization on two different sediment types at three different sites (Ohio and Michigan) *in situ*. Taxa richness, abundance, and % EPT responded negatively to increasing SEM_{Ni}/AVS values, and site differences were observed. Finally, *H. azteca* and *Lymnaea stagnalis* were exposed to a series of tests involving singular or combinations of Ni amendments to water, sediment, and food (stable isotope ^{62}Ni) with dissolved organic carbon (DOC), total suspended solids (TSS) water amendments on two sediments types. Both organisms demonstrated numerous survival, growth, and feeding inhibition effects to these Ni-water and Ni-sediment tests. DOC provided a protective survival, growth, and bioaccumulation (^{62}Ni) effect on *L. stagnalis*. Overall, *H. azteca* and *L. stagnalis* responded similarly with regard to food uptake and trophic transfer of ^{62}Ni, which suggested little ^{62}Ni transfer from food sources.

There are fewer studies and data available for the ecotoxicity of Ni, and these studies help discern Ni bioavailability on a range of aquatic organisms and benthic macroinvertebrate communities. This research has better elucidated the toxicity of Ni in freshwater ecosystems, demonstrating the importance of a range of physicochemical factors on Ni bioavailability, and the relative importance of the three primary exposure routes: water, sediment, and food.

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"…Don't try to explain it, just bow your head
Breathe in, Breathe out, Move on"
Jimmy Buffett

Dedication

To my beautiful family, Jennifer, Ashlynn, and Brenna

Your love moves me, each and every day!

CHAPTER 1 - NICKEL AND OTHER METAL TOXICOLOGICAL EFFECTS

1-0 LITERATURE REVIEW

1-1 *Nickel toxicity in freshwater systems*

Metals enter aquatic environments through both natural sources (rock weathering, volcanoes, forest fires, biogenic processes), and anthropogenic sources (mining, smelting, fossil fuel combustion, and many other processes) (Callendar 2003). Once metals have entered the aquatic environment they can cause ecological impairment of the system. However, metal fate and effects are dependent upon the physico-chemical conditions (i.e. acid volatile sulfides (AVS), organic carbon (OC), carbonates, and iron (Fe) and manganese (Mn) oxides) of the system (Burton et al. 2005b, Di Toro et al. 2005, Gomez and Alvarez 2007). These processes determine whether the metal becomes toxic to biological organisms (Hoang et al. 2004, DeLeebeeck et al. 2008), or forms insoluble complexes with ligands or solids which render the metal unavailable to biological organisms (Goldhaber, 2003).

Nickel is the Earth's 24th most abundant element, its atomic weight is 58.693, and is naturally silver in color (HHS 2004). Sources of nickel in the environment come from nickel mining, nickel manufacturing, solid waste incinerators, oil burning power plants, and coal burning power plants (HHS 2004; Sen Gupta and Bhattacharyya 2008). Nickel is used in the manufacture of stainless steel, jewelry, alloys, and batteries. Nickel is a carcinogen and causes chronic bronchitis, compromised lung function, allergy, skin

irritation, and cancer of the lung, skin, and nasal region are common problems with the use of airborne nickel (Sen Gupta and Bhattacharyya 2008, Green-Pedersen 1997).

Toxicity of Ni usually is associated with its divalent form (Ni^{2+}) that is present in water and sediment porewaters. Ni has been shown to be less toxic than other trace metals such as, cadmium (Cd), copper (Cu), zinc (Zn), lead (Pb), mercury (Hg) (Kallanagoudar and Patil 1997; Doig and Liber 2006; Meyer et al. 2007). Ni ecotoxicological research has experienced less attention over the years, but recently Ni research has seen an increase (Cloran et al. (2010), De Schamphelaere et al. (2010), Nguyen et al. 2011, Costello et al. (2010, 2012)).

The literature has shown that Ni is toxic to aquatic organisms. Meyer et al. (2007) found that *Pimephales promelas* 96 h Ni LC_{50}'s increased significantly (10x) as hardness was increased. They concluded that hardness adjustments could not be applied to activity and concentration of Ni. Kallanagoudar and Patil (1997) found that the fish *Gambusia affinis* also showed similar patterns as hardness increased so did the 96 h LC_{50}'s, suggesting a protective effect from hardness. Study by Klerks and Fraleigh (1997) looked at Ni accumulation in *Dreissena polymorpha* from dissolved and particulate forms. They found that *D. polymorpha* were able to uptake Ni from both forms even though little Ni was associated with particulate fraction (<10%) versus significantly more in the dissolved form (>90%). They concluded that *D. polymorpha* was more affected by the dissolved form than particulate form.

Recently, a number of studies have tested single species and community responses to Ni in laboratory and field studies (Vandegehuchte et al. 2007; Cloran et al. 2010, Costello et al. 2011, 2012, Nguyen et al. 2011). These studies demonstrated the importance of Ni toxicity, and how single species and benthic communities have responded to increasing bioavailable Ni. Community tests using natural sediments spiked with metals (Ni and four others) have been studied by Boothman et al. (2001). Boothman et al. (2001) found no differences in benthic communities between reference sediment and metal spiked treatments. They stated that the simultaneously extracted metal to AVS model (SEM-AVS) did not predict toxicity in lower treatments, but used it to suggest toxicity in the higher treatments. Lee and Lee (2005) found that Ni spiked sediments showed an increase in Ni tissue concentrations in *Neanthes arenaceodentata*, but this was not related to the SEM_{Ni}/AVS model. They also found that this tissue increase was related to Ni in overlying water, but mortality was not related to Ni in the overlying water. Nowierski et al. (2005) found that *H. azteca* accumulated Ni from sediments even when Ca^{2+} concentrations increased. This does not support the sBLM in that metal toxicity is supposed to lessen in the presence of more Ca^{2+} (Di Toro et al. 2001; Di Toro et al. 2005). Nowierski et al. (2005) also found that there was little effect from the overlying water for the Ni body concentrations, and most Ni came from interactions with the sediments. They also state that Ni^{2+} and Mg^{2+} compete more so than do Ni^{2+} and Ca^{2+}. Thus Mg^{2+} should be looked into further when performing future Ni toxicity tests.

Metals are essential for proper health and maintenance of organisms (USEPA 2007), and these are termed 'essential macronutrients' or 'essential micronutrients' (Chapman and Wang 2007). When certain trace metals are not available or are available in low doses, adverse effects on the organism's physiology requirements can occur (USEPA 2007). Chapman and Wang (2000) list some functions for most metals of toxic concern. Cadmium and nickel are two metals where recent research has found that these metals occupy essential physiological requirements and/or functions (Chapman and Wang 2000).

1-2 SEM/AVS, SEM-AVS

Acid volatile sulfides are an important component of anoxic sediments, which can control metal bioavailability due to its ability to bind up free metals in sediments (Di Toro et al. 1996; Rickard and Morse 2005; Burton et al. 2007). AVS is defined as the amount of sulfides present in sediments and these sulfides mostly consist of iron monosulfide (FeS), mackinawite (FeS), pyrrohite (FeS), greigite (Fe_3S_4) (Di Toro et al. 1990; Leonard et al. 1999; Yu et al. 2001). This sediment region is complex and in freshwater systems the AVS layer has a finite thickness (Rickard and Morse 2005). AVS is primarily produced by sulfate reduction (Yu et al. 2001) with sulfur reducing bacteria (SRB) and archaea driving this microbial sulfate reduction process (Goldhaber 2003; Rickard and Morse 2005). As SRB breathe in sulfate (SO_4^{2-}) and respire hydrogen sulfide (HS^-) and CO_2 (Goldhaber 2003). Organic carbon and hydrogen sulfide are

typically found in content and concentrations, since SRB consume OC for energy (Goldhaber 2003). This layer of sediment is black in color due to its sulfide content, and association with pyrite (Rickard and Morse 2005). However, Rickard and Morse (2005) state that field assessment by color alone will not necessarily lead the researcher to the correct conclusion. They point out there are six shades of black, and AVS is bounded above the sub-oxic layer (brownish) and below the pyrite layer (bluish gray) (Rickard and Morse 2005). This sulfide rich component of the sediments reacts with 1 N HCl to produce H_2S (gas), and here is where AVS gets its notoriety as being 'operationally defined' and complex (Rickard and Morse 2005). During this reaction process simultaneously extracted metals (SEM) are analyzed to determine metal concentrations and is where sediments can be determined toxic (Rickard and Morse 2005). The SEM to AVS ratios or numbers is best used to predict if sediments are not toxic.

(1) SEM/AVS < 1 (not toxic)

(2) SEM/AVS > 1 (potentially toxic)

(3) SEM-AVS ≤ 0 (not toxic) (use when AVS concentrations are low)

(4) (SEM-AVS)/foc < 130 μmol/g (not toxic)
(SEM-AVS)/foc 130-3400 μmol/g (uncertain)
(SEM-AVS)/foc > 3400 μmol/g (potentially toxic)

Rickard and Morse (2005) have pointed out that AVS is very complex, and is not as straightforward as some scientists assume. AVS can change with subsurface depth and length (or horizontal area) of these AVS zones (Rickard and Morse 2005; Burton et al. 2007). Rickard and Morse (2005) also suggest that AVS concentrations may change

temporally from days to seasons in the presence or absence of oxygen in overlying waters. De Jonge et al. (2012) found AVS concentrations decreased with depth (0-4 cm), with higher overlying water dissolved oxygen concentrations (90% saturation). Boothman et al. (2001) found that in anoxic sediments AVS decreased in the surficial sediment layer, and increased in the deeper sediment (depth of 10 cm). They also found the similar pattern for SEM concentrations, but noted that SEM concentrations were unchanged below the 2 cm layer. Burton et al. (2007) state that AVS concentrations will vary in different hydrologic areas of streams (i.e. depositional areas = potentially higher AVS, erosional areas = potentially less AVS), and this is dependent on grain size of the sediment and amount of organic matter present. Doig and Liber (2006a) found that sediments with a range of grain sizes (sand, silt, clay) could not predict the amount of AVS present (i.e. higher % of clay did not equate to higher AVS or OM). These results confirm that AVS concentrations did vary spatially (Burton et al. 2007), and temporally (Boothman et al. 2001).

Rickard and Morse (2005) also have shown that dissolved sulfides can change on a diurnal scale, and suggest using caution when applying SEM-AVS models to predict toxicity to biological organisms. Batley et al. (2002) state that SEM/AVS is better at predicting whether a sediment is non-toxic, but falls short in predicting toxicity because of other metal binding compartments (i.e. organic carbon). However, other studies have shown the importance of SEM/AVS ratios for determining toxicity in contaminated sediments (Di Toro et al. 1990; Berry et al. 1996; Lee et al. 2000; Burton et al. 2007).

There are numerous studies that indicate the importance of AVS and its affect on bioavailability of metals in sediments (Di Toro et al. 1990; Ankley et al. 1991; Berry et al. 1996; Lee et al. 2004; Burton et al. 2005).

Berry and co-workers (1996) state that metal sulfides have lower solubility constants than iron and manganese sulfides. These metal sulfides have lower solubility than iron and manganese sulfides (Table 1), and NiS has the highest solubility constant of all the trace metal sulfides (Di Toro et al. 1990). When SEM/AVS ratios are > 1 then metals should appear in the porewater in order of highest solubilities first (i.e. Ni first) (Berry et al. 1996). These metal sulfides displace iron and manganese sulfides to form a greater insoluble sulfide, thus making them essentially non-bioavailable (Di Toro et al. 1990). In addition, the low solubilities of metal sulfides will result in lower metal concentrations in the porewater (Simpson et al. 1998).

Table 1-1. Metal sulfide solubility constants.
Modified from Di Toro et al. 1990

Metal sulfide	$\log K_{sp,2}$	$\log K_{sp}$	$\log \alpha$ pH = 7.6	$\log \alpha$ pH = 8.2	$\log(\alpha K_{sp})$ Average
MnS	−0.40	−19.15	0.13	0.13	−19.02
FeS(am)	−3.05	−21.80	0.10	0.12	−21.69
FeS	−3.64	−22.39	0.10	0.12	−22.28
NiS	−9.23	−27.98	0.11	0.17	−27.84
ZnS	−9.64	−28.39	0.12	0.14	−28.26
CdS	−14.10	−32.85	1.50	1.50	−31.35
PbS	−14.67	−33.42	1.12	1.32	−32.20
CuS	−22.19	−40.94	0.50	0.92	−40.23
HgS	−38.50	−57.25	15.10	15.10	−42.15

In freshwater sediments when no other binding phase (DOC, TOC, carbonates, etc) is present, and the SEM/AVS ratio is greater than 1, the sediment then fits the model and may be toxic (Berry et al. 1996). However, this is not realistic of natural conditions, and is a practice in theory. The binding phase of sulfides is a complex feature of sediments, but has been studied and modeled extensively to provide additional insight to metal sediment toxicity. Understanding this phase will enable future metal sediment toxicity studies to be designed properly.

1-3 Total and dissolved organic carbon (TOC and DOC)

The use of natural organic matter (NOM) has received much attention in aquatic metal research (Ma et al. 2002; Kashian et al. 2004; Kramer et al. 2004; Doig and Liber 2006), and this attention will remain strong as long as the Biotic Ligand Model (BLM) continues to be revisited (Di Toro et al. 2001). USEPA (2005) states the main binding phases in sediments includes organic carbon. USEPA (2005) states that dissolved metals in sediments are easily adsorbed to DOC which may not be bioavailable. In oxic freshwater sediments a common phase of organic carbon is in the particulate form, and porewater is DOC (possibly colloidal) (USEPA 2005). The particulate form has a large amount of sorptive area allowing dissolved metals to adsorb on to the outside layer (USEPA 2005). The SEM-AVS difference normalized to fraction of organic carbon (equation 4) has been shown to provide a better model for predicting toxicity in sediments (Di Toro et al. 2005). There are however, uncertainty bounds for this

normalized model. The USEPA (2005) states that toxicity is possible when (ΣSEM-AVS)/foc is >3,000 µmol/gOC, and not toxic when OC concentrations are below 130 µmol/gOC and uncertainty when OC concentrations are between 130 and 3,000 µmol/gOC (USEPA 2005). Mahony et al. (1996) states that even when no AVS is present, metal concentrations may be below the sediment quality guidelines (SQG) because of the amount of OC present in the sediments.

In water-only tests the most common use of dissolved organic matter (DOM) is in the dissolved phase, and has been shown to reduce the toxicity of metals (Cd, Cu, Ni, Ag) and other contaminants (Voets et al. 2004; Kramer et al. 2004; Glover and Wood 2005; Doig and Liber 2006). DOM has been quantified as dissolved organic carbon (DOC) in the previous studies, and both are measure of carbon in the system (Martino et al. 2003; Doig and Liber 2006). Martino et al. (2003) found that Ni adsorption to DOM was rapid (few hours) and equilibrium was achieved in ~ 24 h. Martino et al. (2003) states that the amount of river DOC was the only limiting factor in the adsorption of nickel. Doig and Liber (2006) found 48 h LC_{50} for *Hyalella azteca* in all DOM (humic acid and fulvic acid) tests combined was 14 mg/L of Ni. They found that increasing the DOC from 1-30 mg/L had no effect on the *H. azteca* 48 h LC_{50}. Additionally, a 96 h was not affected by DOC levels and the LC_{50} value was 4.2 mg/L. They found that *H. azteca* tissue were significantly different in all but two of the Ni and DOC treatments. Demonstrating a strong correlation between the tissue concentrations and free nickel concentrations, and the free nickel concentration was affected by the amount of DOC in the water (Doig and

Liber 2006). However, there appears to be no protective effect of DOC (at range tested) from Ni on *H. azteca* LC_{50}'s and body burden concentrations. The authors suggested that Ni:DOC ratios were off, and that the free nickel present may in fact be speciated to $NiCO_3$ at pH values >8.

A study by Glover and Wood (2005) examined Ag toxicity on *Daphnia magna* with the addition of DOM. The authors showed that during the 24 h accumulation tests NOM (AHA or NRN) provided significant differences in silver accumulation in *D. magna*. These differences were seen in type of NOM used, with AHA showing the lowest silver accumulation in Daphnia regardless of concentration. The ability of NOM (DOC) to attenuate metal toxicity and bioaccumulation in different organisms appears to an important ligand. This research area is open uses with metals and metalloids, and use of additional organisms (feeding styles) will provide insight to metal bioavailability.

A very interesting study found that DOC levels have increased significantly in United Kingdom over a 12 year period (Worrall et al. 2004). Over a 190 sites were surveyed and they have hypothesized that increasing temperatures over the past decades are driving microbial rates thus increasing the amount of DOC released during this processes (Worrall et al. 2004). Given the global climate dialogue and slight temperature increases to date, this could have a possible effect on DOC levels elsewhere, and also drive metal criteria.

1-4 Iron (Fe) and Manganese (Mn) oxides

USEPA (2005) states that Fe and Mn are main partitioning phases of metals in both anoxic and oxic sediments and these oxides quickly scavenge free metals. Sundby (1994) states that trace metals are taken up by Fe and Mn oxyhydroxides when pH increases, and when these oxyhydroxides are exposed to reduced pH conditions metals may then be released. Chapman et al. (1998) stated that iron oxyhydroxides (FeOOH) and manganese oxyhydroxides (MnOOH) are part of the key binding phases in oxic and anoxic sediments.

$$FeOOH + Me^{2+} = FeOOMe^{+} + H^{+} \quad (5)$$

$$MnOOH + Me^{2+} = MnOOMe^{+} + H^{+} \quad (6)$$

The above reactions bind metals and form a precipitate that is insoluble and not bioavailable. Peng et al. (2004) found that gut contents of an oligochaete were higher in sediments that had no Fe and Mn oxides. Di Toro et al. (1996) stated that when FeS is oxidized FeOOH is produced. Under these conditions once metals are bound to FeS and it is subsequently oxidized, metals will be released and the product FeOOH will scavenge the released metals.

Prasad et al. (2006) found that Cd, Zn, Cu, Pb had high affinities to FeOOH and MnOOH. They also found that Fe and Mn were affected by host of small grain sizes (< 67 μm). They found that the highest concentrations of Fe and Mn were between depths 2-8 cm, dropped substantially between depths of 8-10 cm, and then started to increase again. Gomez-Alvarez et al. (2007) found that in sandy-gravel and silty-sand sediments Fe and Mn oxides were still an important portioning phase, much more than the

OM/sulfide phase. These studies discuss the importance of Fe and Mn oxides, and how metals partition onto these and form insoluble complexes which are not bioavailable. There are conditions (pH and redox) when these Fe and Mn phases can release metals, and understanding these will enable better study design.

1-5 Redox effects

Redox is another sediment parameter that is important to the bioavailability of metals. Allen (1995) states that redox in natural aquatic systems are driven by the oxidation of OC by microbial community, with oxygen and sulfur being the most important electron acceptors. Little OC equates to oxic condition and sufficient amounts of OC will mean anoxic conditions (Allen 1995). Whenever metals are released (flux) either at the sediment-water interface or within sediments. Sundby (1994) states that flux is usually driven by reduction, and reducing conditions favor sulfide reactions. It is here that AVS may dominate the reaction and bind the released metals into insoluble metal sulfides (Sundby 1994).

When anoxic sediments are oxidized, metals are released (Miao et al. 2006), and this flux allows metals to either become bioavailable or to be scavenged by other partitioning factors (e.g. AVS, FeOOH, MnOOH, carbonates, OC). De Jonge et al. (2012) found that by increasing overlying water oxygen concentration that Eh decreased with depth and time. Sundby (1994) states that reductions of Fe (III) to Fe (II) and Mn (IV/III) to Mn (II) are controlled by oxyhydroxides and complexation. Decomposition of

OM can drive the reduction of Fe (III) and reduction rate increases with decreasing pH (Sundby 1994). In anoxic sediments, Mn-oxyhydroxides are reduced easier than the Fe-oxyhydroxides (Sundby 1994). Metal solubility can change with depth (Miao et al. 2006), and depending on the partitioning phase available will determine its solubility. Miao et al. (2006) stated that Ni, Cd, Zn, Cu, and Cd are redox sensitive, and these are affected by changes in the redox-potential of sediments. Miao et al. (2006) showed that in sediments as pH declined the Eh increased, and vice versa. They also found that Fe and Mn concentrations decreased with oxidation and increased with reduction. In these sediments Miao et al. (2006) found that when Fe and Mn are oxidized in the oxic layer they are insoluble, and when reduced these metal oxyhydroxides are more soluble (i.e. mobile).

1-6 Hardness and carbonates

Evangelou (1998) emphasizes the importance of understanding the difference between dissolved and total metals. Total metals include the whole water sample while dissolved metals can be obtained by filtering out particles either 0.45 or 0.20 μm filters. Evangelou (1998) states at pH ranges of 7-10 iron is $FeCO_3$ and manganese is $MnCO_3$ dominate, and both are also dependent upon the pE activity. Hardness is the measured amount of Ca^{2+} and Mg^{2+} in a system and is reported as mg/L of $CaCO_3$. Hardness has the ability to attenuate metal toxicity in a system, and this is in part due to the competition of binding sites at the gill (fish and invertebrates) with divalent metals.

Numerous studies have showed a hardness protection effect with both invertebrates and fish (Meyer et al. 1999; Pyle et al. 2002; Keithly et al. 2004). These hardness protective effects are best described in the BLM (Di Toro et al. 2001) where toxicity from metals are predicted in the presence or absence of ligands.

Hamelink et al. (1994) suggest that metals will form complexes with carbonates over a range of pH conditions. However, Hoang et al. (2004) stated that the BLM might not predict Ni toxicity at higher pH, and $NiCO_3$ may be more bioavailable than once thought, thus reasons why sometimes metals become more toxic at higher pH's (Hamelink et al. 1994). Yu et al. (2001) found that excess SEM (after all AVS used) bound to OM and carbonates over oxides, and this was apparent at depths down to 15-20 cm. It appears that Fe, Mn, carbonates, and OM are limited to metal partitioning as depth increases (i.e. deeper sediment = lower binding activity) (Yu et al. 2001).

Considering hardness and carbonate variables is important to the design of any metal research project. Differences in toxicity are possible when hardness levels are varied, and carbonate species present. Thus higher hardness and carbonate speciation can affect the bioavailability of the metal.

1-7 Adsorption to solids

Trace metals prove to be environmentally challenging because of their persistent nature and toxicity to organisms (Sen Gupta and Bhattacharyya 2008). Sen Gupta and Bhattacharyya (2008) state that adsorption immobilizes metals from their aqueous phase,

and allows them to settle out when the clay particles deposit. Clay-metal interactions follow two processes, either adsorption or cation exchange (CEC), or even both can occur simultaneously (Abollino et al. 2008; Sen Gupta and Bhattacharyya 2008). Clays are alumniosilicates which are important components of the soil (Sen Gupta and Bhattacharyya 2008), and enter aquatic systems through runoff events from agriculture practices, or urban construction (Burton 1991). Common CEC process with clays happens when their major cations (Si^{4+} and Al^{3+}) are substituted with lower valence cations (i.e. divalent metals) (Abollino et al. 2008). Three common clays used in research purposes are montmorillonite, kaolinite, and vermiculite (Albino et al. 2008; Sen Gupta and Bhattacharyya 2008).

Sen Gupta and Bhattacharyya (2008) found that the surface area for montmorillonite (18.7 m^2/g) was 5x greater than kaolinite (3.1 m^2/g), and when calcined (773 K) surface area increased for both, 19.8 m^2/g and 3.8 m^2/g respectively. Sen Gupta and Bhattacharyya (2008) found that with increasing pH the metals Cd, Ni, and Pb were increasingly adsorbing to kaolinite and montmorillonite to a pH of 10. Abollino et al. (2008) recorded similar results with montmorillonite and vermiculite, as pH decreased, metal adsorption decreased. They also found that montmorillonite adsorbs more metals than kaolinite at a rate of 17x, but kaolinite adsorbs more Ni than Cd, and montmorillonite adsorbs more Cd than Ni. The explanation for pH differences in clay-metal binding is that there are more H_3O^+ ions at low pH and divalent metals cannot compete, but as pH increases the H_3O^+ are released thus freeing up binding sites for

metals (Sen Gupta and Bhattacharyya 2008). In the Abollino et al. (2008) study, most of the metals tested (Cd, Cu, Mn, Ni, Pb, Zn) were near 100% sorbed to montmorillonite at pH greater than 8.0. Sen Gupta and Bhattacharyya (2008) found that when clay was loaded in high concentrations the amount of metal ions decreased that were adsorbed. The opposite was found when metal ion concentrations were increased; more metals were adsorbed to the same amount of clay.

The reaction time was fast for metals (Pb, Cd, Ni) interacting with clays (40 min), and equilibrium was achieved for Ni at 180 min (Sen Gupta and Bhattacharyya 2008). Even though montmorillonite is a 2:1 octahedral sheets and kaolinite is 1:1 sheet, there was no difference in metal equilibrium times. As temperature increased (29.8-39.8°C) metal adsorption to clay decreased, and was explained by solubility of metals increase with increasing temperature (Sen Gupta and Bhattacharyya 2008). Abollino et al. (2008) found that metal adsorption to clay was significantly reduced in the presences of organic ligand (e.g. NTA and EDTA). The metals preferred these organic ligands over the clays, and they state that the complex becomes more stable with increasing hydrocarbon chain length. Only exception was Mn^{2+} which still had an affinity to clay in the presence of organic ligands (Abollino et al. 2008). Abollino et al. (2008) found that montmorillonite adsorbed metals (Ni>Mn>Zn>Cu>Cd=Pb) and vermiculite (Mn>Ni>Zn>Cd>Cu>Pb) in order of atomic weight, suggesting that metals with higher atomic mass had a harder time diffusing inside the clay lattice.

1-8 Dietary uptake of metals

Metals uptake by organisms occurs from three different routes of exposure: ingestion (food), absorption (gill epithelial), adsorption skin (consumed and transferred to higher trophic levels) (Chapman 2008). Bioconcentration factors (BCFs) from water-only exposures, and bioaccumulation factors (BAFs) from field exposures are ways to determine metal tissue concentrations (Chapman 2008). As Chapman (2008) and McGeer et al. (2003) point out, there are limitations to the uses of BCFs and BAFs with metals in aquatic organisms in risk assessment because metals do not always bioaccumulate as expected. McGeer et al. (2003) discussed the alternative use of accumulation factors (ACFs) to possibly bridge the gap between BCF and BAF. They state that their model still needs further research and does not stand alone as a means to replace the BCF (McGeer et al. 2003).

However, there are numerous studies that used metal tissue concentrations as a means to determine metal toxicity (Chen and Mayer 1999; Lee et al. 2000; Ball et al. 2006; Wilding and Maltby 2006). Study by Lee et al. (2000) looked at Cd, Ni and Zn accumulating in the four different organisms with different feeding strategies (filter feeder, facultative deposit-feeder, surface deposit-feeder, deep sediment deposit-feeder). They found that all bioaccumulated metals under conditions with and without AVS. However, the exception was the deep sediment feeder showed an AVS protective effect, mainly because this polychaete does not aerate its tube (Lee et al. 2000). Aerating its tube (bioturbation) can lower concentrations of AVS by oxidation, and make metals

bioavailable. Studies by Ball et al. (2006) and Wilding and Maltby (2006) showed that metals are able to accumulate in food (leaf and algae), and amphipods are showing growth and survival effects. Courtney and Clements (2002) showed that grazing mayflies exposed to Zn contaminated biofilms were bioaccumulating the metal from the food source, and growth was affected during these 7 d exposures.

These studies show the importance of metals in dietary uptake, and as Wilding and Maltby (2006) state there is a lack of research and regulatory direction in this area. Wilding and Maltby (2006) indicate that feeding and feeding rates have an effect on survival, growth, reproduction, and all of these have a compounding effect on populations.

Metals in food and subsequent uptake by organisms in sediments and water-only exposures should continue to be examined (Wilding and Maltby 2006). This area of metal accumulation from diet is gaining momentum, and optimistically may start showing up in regulatory efforts.

1-9 Biotic ligand model (water and sediments)

The BLM stands out as an important paper to understand the mechanisms driving metal toxicity on biological organisms (Di Toro et al. 2001). Determining whether or not these metals are bioavailable is an arduous task. The BLM (Di Toro et al. 2001) was adapted from the gill surface model work by G. Pagenkopf in the early 1970's. Di Toro et al. (2001) state that metal toxicity is not just driven by aqueous concentration, but

rather a complex mixture of metal bound ligands. Only when these metal concentrations in water and on complexed ligands exceed some threshold level will the organism experience toxicity (Di Toro et al. 2001). They state organism toxicity to metals will occurs when the free metal affects the organisms' physiology, thus creating the metal-biotic ligand (Me-biotic ligand). In fish this occurs at the gill site, and metals affect the gill Na^+ transfer. In water-only systems these ligands are competing for sites on the gills, and it is here where toxicity can occur either through entry to the organisms' body, or in the case of Ni, complete damage to the gill and suffocation (Chapman and Wang 2000).

Di Toro et al. (2001) state that DOC (humic and fulvic acid) is an important ligand. Other competing cations are Na^+, H^+, Ca^{2+}, complexing inorganic ligands are $MeOH^+$, $MeHCO_3^+$, $MeCl^+$, and complexing organic ligands are Me-DOC (Di Toro et al. 2001). When DOC and metals are present, hardness plays a role in metal complexes to DOC. They found that as Ca^{2+} increases this causes a decrease in DOC metal complexes because both are divalent and competing for binding sites. In addition, when pH decreases the number H^+ increases and these can compete with metals for binding sites to DOC (Di Toro et al. 2001). The BLM can be used to determine mortality to a population through the LC_{50} or EC_{50} (Di Toro et al. 2001).

In sediments the BLM (Di Toro et al. 2001) was not applicable because there are other partitioning phases for sediments that must be considered. The Di Toro et al. (2005) sediment BLM expands on the equilibrium partitioning (EqP) which can predict the lack of toxicity (i.e. ΣSEM/AVS model). The sediment BLM (sBLM) considers OC

in sediments as the key partitioning phase, but also because OC is commonly measured in the other sediment studies. The sBLM uses the Windmere Humic Aqueous Model (WHAM) and the biotic ligand model (Di Toro et al. 2001). One reason the model works is because porewater chemistry are ignored (i.e. pH and DOC). However, it is important to note that other studies have demonstrated that FeOOH and MnOOH are important to binding metals (Prasad et al. 2006; Gomez-Alvarez 2007, Costello et al. 2011), as well as carbonates (Lin et al. 2001; Yu et al. 2003) even in the presence of OC and AVS.

As of sBLM publication date, the authors state that the sBLM is limited on *D. magna* data for spiked sediments. Therefore, application of sBLM to spiked sediment tests is more complicated and vague. In addition, the pH drops when metals are added to sediments, and they suggest monitoring porewater pH during these types of tests (Di Toro et al. 2005). For use in spiked sediment tests the sBLM appears to be a work in progress, and future spiked tests should monitor all available parameters especially porewater pH. The BLM and sBLM just affirm that many factors are work when determining metal bioavailability, and with sediments this becomes even more complexed. Di Toro et al. (2005) sBLM appears to be set for further validation with additional partitioning phases (Fe, Mn, carbonates, pH) to be incorporated later.

2-0 GAPS IN THE LITERATURE

2-1 AVS and Ni spiked sediments

The Di Toro et al. (1990) study with Cd-spiked sediment observed a yellow precipitate and hypothesized that dissolved sulfide concentrations in porewater were high and causing this complex to precipitate out. This was not the case and the measured levels of dissolved sulfide in reference sediments were low, and the authors hypothesized that by adding Cd to the sediments this caused a reaction with FeS and subsequently released dissolved sulfides.

When Cd is added to aqueous phase:

$$Cd^{2+} + FeS(s) \rightarrow Cd^{2+} + Fe^{2+} + S^{2-} \qquad (7)$$

Result when Cd concentration exceeds the solubility of cadmium sulfide:

$$Cd^{2+} + FeS(s) \rightarrow CdS(s) + Fe^{2+} \qquad (8)$$

Alternatively, could be written:

$$Me^{2+} + FeS(s) \rightarrow MeS(s) + Fe^{2+} \qquad (9)$$

There is a gap in the literature with Ni spiked sediments, and measuring dissolved sulfide concentrations in reference sediment with high AVS. Since Ni appears to be less toxic than other metals and behaves differently in organism bioaccumulation (Doig and Liber 2006; Meyer et al. 2007) it would be interesting to see if Ni has the same affect on sediments with high AVS. If more sulfides were present in the spiked sediments this would affect the metal availability, and could alter AVS readings in each sediment treatment. If more than one concentration is used, and AVS is measured in all treatments,

then the amount of sulfide in each treatment may be modeled with the amount of Ni spiked.

2-2 DOM and Ni toxicity in water-only experiments

The amount of DOC has been shown to reduce toxicity of metals in sediments and water exposures (Doig and Liber 2004, 2006), but no research has been devoted to nickel and DOC in water, sediment, and food combination exposures. This data could provide valuable insight to sediment toxicity models and incorporated into the BLM (Di Toro et al. 2001), and for understanding the role DOC has on other toxicological endpoints (growth, bioaccumulation). Di Toro et al. (2001) state the lack of nickel research in general, could provide the framework for nickel BLM development, and help predict the mode of toxicity in aquatic species. DOC and Ni studies could be performed with a host of organism, but more specifically the indigenous *Isonychia spp., Stenonema spp.*, and *P. herricki.* Using the indigenous organisms would provide an ecologically relevant theme and would likely mimic what these organisms experience in natural systems during storm events. No Ni toxicity data is available on these organisms, and incorporating DOC would further the BLM.

2-3 Dietary uptake of Ni in indigenous organisms

Dietary uptake of metals is gaining popularity and incorporation of this route of exposure needs applied metal bioaccumulation (Chapman 2008). There are fewer studies

looking at the effects of metal contaminated food (Courtney and Clements 2002; Ball et al. 2006; Wilding and Maltby 2006) and their role in metal toxicity to aquatic organisms. One approach could be to colonize tiles with periphyton (Courtney and Clements 2002) in a clean stream, and then expose the tiles to a metal solution (Ni) and allow grazers (*P. herricki, Stenonema spp*.) to feed. Many approaches to this type of design would allow the use of survival, growth (head capsules widths, lengths, weights, exuvia), and bioaccumulation. Disadvantages are that contaminated food could be avoided (Ball et al. 2006). There are also concerns with whether metals are adsorbed to the periphyton or assimilated by the living cell (Ball et al. 2006).

2-4 Hardness effects on organisms in water-only experiments

Hardness protective effects on aquatic organisms (fish and insects) have been shown in a number of studies (Meyer et al. 1999; Pyle et al. 2002; Keithly et al. 2004). However, taking this protective effect to sediment toxicity could provide a novel approach to understanding toxic effects in Ni spiked sediments. Burton (1991) stated that during storm events rain water entering the streams drops the hardness levels, and these drops also make it to the sediments. A series of experiments would have to be run to see if during these series of water changes the porewater hardness is reacting to the overlying water. Diffusive forces may allow this experiment to work.

2-5 BLM and spiked sediment parameter measuring

Di Toro et al. (2005) stated that the sBLM is a work in progress because of very limited data available for pH, Fe, Mn, OC, in metal spiked sediment tests. This gap in the literature could easily be filled with more spiked sediment test where porewater pH measurements and other partitioning phases (Fe, Mn, carbonates) are measured. The sBLM for spiked sediments is warranted, and without more studies following these recommendations, the sBLM will not be applied to this area of research for some time.

2-6 Whole body vs. tissue concentrations of metals in indigenous organisms

Sola and Prat (2006) examined the lingering effects of metal contamination in rivers and used the aquatic insect *Hydropsyche spp.* as a surrogate for whole body metal accumulation from a spill. The study was trying to find an alternative approach to tissue-accumulated metals because previous methodology was laborious. They hypothesized that whole body burden is more indicative of site contamination.

Whole body burden would be an interesting topic, and provides an ecological relevant answer to how much of a metal is being bioaccumulated by an organism during metal exposures to water and sediment. Sola and Prat (2006) found differences in metal accumulation (Cu, Zn, Pb, and Cd) between the sites, and at all sites metal concentrations in *Hydropsyche* whole body concentrations were significantly higher than the control site. Correlations between *Hydropsyche* metal body concentrations (Cu, Zn, Cd, Ti, and Sb) were positive with water and sediment concentrations. They concluded that higher metal content in *Hydropsyche* revealed lower benthic community scores.

Incorporating bioaccumulation into spiked sediment studies using caged *in situ* exposures with indigenous aquatic insects (Custer et al. 2006) would allow for discerning metal accumulation in differing sediment concentrations. Functional feeding group (filterers, scrapers, or predators) or habits (burrower, clinger, or swimmer) would be an interesting angle to look at whether one type accumulates more Ni. This approach would add an additional line of evidence to weight of evidence for determining if Ni spiked sediments are toxic and does Ni bioaccumulate in organisms.

Nickel bioavailability and toxicity to aquatic organisms was examined in a host of systems, with different species, varying physico-chemical conditions, and the objectives and hypotheses of my research were as follows:

Chapter 2: Objective: Investigate the relationships between Ni sediment concentration, bioavailability, and toxicity to aquatic macroinvertebrates in a streamside flow-through mesocosm. Sediment Ni toxicity was determined by transplanting macroinvertebrate communities and exposing *Hyalella azteca, Chironomus dilutus, Tubifex tubifex*, *Isonychia* spp., and *Psephenus herricki* in the mesocosm system. Changes in AVS, total Ni, SEM-Ni, total Fe, total Mn, organic carbon, porewater dissolve organic carbon (DOC), and sediment pH were monitored in both sediment types at 4, 7, and 17 wks.

Hypothesis: Macroinvertebrate community toxicity will be attenuated with higher AVS and OC sediments, and with time.

Chapter 3. Objective: The objective of this study was to examine how field collected benthic macroinvertebrate communities responded to a gradient of Ni spiked in two different sediment types in a streamside mesocosm, and if Ni bioavailability is being affected in these different sediments.

Hypothesis: Macroinvertebrate communities will respond negatively to increasing Ni, and sediment with low AVS and OC content will have most bioavailable Ni over time.

Chapter 4. Objective: The objectives of this study were to compare four indigenous aquatic insects with two USEPA surrogate organism responses (lethal and sublethal) to Ni-spiked sediments in flow-thru exposures. Also, an abbreviated method for simultaneously extracted metal (SEM_{Ni}) was compared to the standard USEPA SEM/AVS method.

Hypothesis: *Isonychia spp*. < *P. herricki* < *Stenonema spp*. < *A. verticis* in Ni sensitivity, and *Isonychia spp*. growth will be most sensitive sublethal endpoint for all indigenous insects.

Chapter 5. Objective: The objectives of this study were to evaluate benthic macroinvertebrate community responses in the presence of Ni-spiked sediments, while considering the physical characteristics of two sediment types and the three sites. Also determine if benthic invertebrate communities prefer larger grain size sediments over smaller grain size sediments by differences in diversity indices and benthic metrics.

Hypothesis: Macroinvertebrate colonization will be negatively affected by increasing Ni, and also site differences will be observed. Benthic macroinvertebrate communities will colonize larger grained substrates over smaller grained substrates.

Chapter 6. Objective: The objectives of this study were to determine whether *Lymnaea stagnalis* and *Hyalella azteca* would respond negatively and/or bioaccumulate Ni during a series of Ni amendments to water, sediments, and food (either in singular or in combination), while receiving water changes with TSS and DOC on two different sediment types.

Hypothesis: Whole body Ni accumulation from Ni labeled food will be greater in *L. stagnalis* than *H. azteca*. The DOC and TSS amendments will not be protective of Ni toxicity and Ni bioaccumulation during Ni sediment toxicity tests.

CHAPTER 2 – EXAMINING THE EFFECTS OF SEDIMENT NICKEL IN A STREAMSIDE MESOCOSM (2007)

By

Kevin W. Custer[1], G. A. Burton, Jr[1,2], W. K. Taulbee[1,2], P. Anderson[1], C. Cloran[1], J. Kochersberger[1], and C. Schlekat[3]

[1]Wright State University
Department of Earth & Environmental Sciences
3640 Colonel Glenn Highway
Dayton, Ohio 45435

[2]University of Michigan
Natural Resources and the Environment
Ann Arbor, Mi

[3]Nickel Producers Environmental Research Association
Durham, NC

1-0 ABSTRACT

Disclaimer: This chapter was written in collaboration with Drs. G. Allen Burton Jr., W. Keith Taulbee and I for submission as final report to Nickel Producers (NiPERA). Some of this work has been included in the publication by Costello, D.M., G. A. Burton, C.R. Hammerschmidt, and W.K. Taulbee. 2012. Evaluating the Performance of Diffusive Gradients in Thin Films (DGTs) for predicting Ni Sediment Toxicity. Environmental Science and Technology, 46: 10239-10246.

The bioavailability of nickel (Ni) in freshwater sediments was evaluated in streamside mesocosms, focusing on benthic macroinvertebrate population and community responses. Two different sediment types were used: depositional sediment (Warden Ditch (WD)) that was high in acid volatile sulfides (AVS), organic carbon (OC), and erosional sediment (Mad River (MR)) that was low in AVS and OC. Each sediment type was spiked with a dilution series of Ni concentrations. The WD total Ni concentrations at time zero were 9680, 5180, 2240, 1290, 658 mg/kg and MR concentrations were 1030, 692, 278, 140, 80 mg/kg, and seeded with macroinvertebrate communities. Reference and Ni spiked sediments were deployed with reference stream benthic macroinvertebrate seeding on 1-Aug-07, and sampled at 4, 7, 8, and 17 wks, with exposures ending 24-Nov-07 (17 weeks). The results showed both significant differences and similarities in Ni fate and effects between the two sediments. In the MR sediments, Ni flux (i.e., loss from sediments) was greatest (9-89 % of total Ni). Ni loss in WD sediments was less over time (11-47 %). Diffusive gradient in-thin-films (DGT) analysis of labile Ni further documented the flux of Ni out of the sediments to overlying water and periphyton. Overall, the magnitude of the Ni loss from spiked sediments could be

explained by the relationships between Ni concentrations and the number of binding/complexation sites available in the sediments. The SEM_{Ni}/AVS ratios were high in MR suggesting that all the Ni treatments may be toxic (SEM_{Ni}/AVS > 65). However, WD sediments had SEM_{Ni}/AVS ratios ranging from below 1 to 23.2. As the study continued, (SEM_{Ni}-AVS)/foc increased slightly in the WD sediments (-640 – 2146 µmol/gOC). The MR treatments all had high (SEM_{Ni}-AVS)/foc > 1476-13016 µmol/gOC. The indigenous community revealed decreases in both the total numbers of numerically dominant invertebrate taxa and total invertebrates with increasing Ni. Macroinvertebrate density and total taxa were negatively correlated to SEM_{Ni}/AVS and (SEM_{Ni}-AVS)/f_{OC}. These relationships were not observed within the periphyton layer, which had the highest invertebrate numbers in the highest Ni treatments. There was a significant positive relationship between manganese (Mn) and Ni in the periphyton layer, suggesting the complexation of Ni with Mn oxyhydroxides in this highly oxic substrate and Ni was not bioavailable. Both AVS and OC mitigated Ni toxicity, as evidenced by the significant negative relationships between invertebrate densities and both SEM_{Ni}/AVS and (SEM_{Ni}-AVS)/f_{oc}. However, results also demonstrated that the (SEM_{Ni}-AVS)/foc model of Ni bioavailability alone does not fully explain toxicity to benthic macroinvertebrates. Benthic invertebrate responses to Ni based on SEM_{Ni}-AVS)/ foc cannot be interpreted in isolation; rather, they are secondarily dependent on sediment type (i.e. particle size). Benthic invertebrates demonstrated a pronounced preference for colonizing the sandier, low AVS, MR sediments, even though overall Ni bioavailability,

as predicted by the (SEM_{Ni}-AVS)/foc model, was generally higher in those sediments than in the WD sediments. The formation of Fe and Mn oxyhydroxides, presence of periphyton, and hard water all likely contributed to the lack of Ni toxicity.

2-0 INTRODUCTION

Waterborne Ni is an important environmental contaminant that is most commonly associated with mining, smelting, refining, alloy processing, scrap metal reprocessing, and waste incineration (Eisler 1998). Relative to the other divalent metals, Ni is both generally less toxic and also generally less well studied (Kallanagoudar and Patil 1997; Keithly et al. 2004; Doig and Liber 2006; Meyer et al. 2007). Where mechanisms of waterborne Ni toxicity have been investigated, it has been shown to result from Mg^{2+} antagonism in *Daphnia magna* (Pane et al. 2003a), and to act as a respiratory toxicant to rainbow trout *Oncorhynchus mykiss* (Pane et al. 2003b).

Ni is similar to other divalent metals in that its bioavailability and toxicity in sediments is mitigated by concentrations of acid volatile sulfides (AVS), Fe- and Mn-oxyhydroxides, organic carbon (OC), and water hardness (Keithly et al. 2004; Di Toro et al. 2005; Lee and Lee 2005; Vandegehuchte et al. 2007; Nguyen et al. 2011). In a 28 day sediment toxicity test using the marine polychaete *Neanthes arenaceodentata*, Lee and Lee (2005) observed growth rate to be negatively correlated to SEM_{Ni}-AVS. In a laboratory study of Ni bioaccumulation from spiked sediments in the oligochaete *Lumbriculus variegates,* bioaccumulation of Ni was most accurately predicted by

(SEM_{Ni}-AVS)/f_{OC}, and was not correlated to total sediment Ni (Vandegehuchte et al. 2007). However, in both of these studies, toxicity could also be predicted by the concentration of free Ni [Ni^{2+}] in the overlying water.

Field exposures are needed to provide more realistic Ni thresholds to aquatic organisms. The use of *in-situ* colonization trays have been used to successfully investigate benthic invertebrate responses to metal toxicity in several long terms field studies (Boothman et al. 2001; Burton et al. 2005; Costello et al. 2011, Nguyen et al. 2011). In this design, field collected sediments that differ in chemical properties (e.g. AVS, OC, Mn, Fe) are spiked with metals in the laboratory and returned to the field. Burton et al. (2005) utilized this approach to demonstrate that benthic macroinvertebrates in four European water bodies responded in accordance with the (SEM-AVS)/f_{OC} model of bioavailability, in that abundance and diversity declined above bioavailability thresholds (defined as SEM_{Zn}-AVS, SEM_{Zn}/AVS, SEM_{Zn}-AVS/f_{OC}) hypothesized by Di Toro et al. (2005). Similarly, in a study investigating the effects of AVS and OC on macroinvertebrate responses to Ni, Nguyen et al. (2011) observed declines in abundance and diversity measures above Ni thresholds predicted by the (SEM-AVS)/foc model of metal bioavailability. In both studies, invertebrate responses were not correlated with total metal concentrations. These *in-situ* field exposures are not without their experimental confounds.

An alternative approach that provides both the realism of a field exposure with the added control of the laboratory are field mesocosm studies, which have been used successfully to investigate causal relationships between stressors and relevant biological responses in numerous studies (Clements 2004; Clark and Clements 2006). In order to investigate relationships between Ni sediment concentration, bioavailability, and toxicity to aquatic macroinvertebrates, we conducted a 17 week field exposure from July 31 – 27-Nov-07 within a streamside flow-through mesocosm. Effects of a Ni concentration gradient on benthic invertebrate responses were evaluated using two field-collected sediments with differing sediment characteristics (AVS, OC, Fe, and Mn). Sediments spiked with Ni were added to colonization trays, placed within the mesocosm, and removed periodically throughout the study to evaluate sediment chemistry as well as biological responses to both total and bioavailable Ni as predicted by the SEM-AVS model of metal bioavailability. In addition, the possibility of acute Ni toxicity was investigated using a series of caged *in-situ* exposures to three surrogate species (*Hyalella azteca, Chironomus dilutus, Tubifex tubifex*) and two indigenous species (*Isonychia* spp., and *Psephenus herricki*).

2-1 Objective: Investigate the relationships between Ni sediment concentration, bioavailability, and toxicity to aquatic macroinvertebrates in a streamside flow-through mesocosm. Sediment Ni toxicity was determined by transplanting macroinvertebrate communities and exposing *Hyalella azteca, Chironomus dilutus, Tubifex tubifex*,

Isonychia spp., and *Psephenus herricki* in the mesocosm system. Changes in AVS, total Ni, SEM-Ni, total Fe, total Mn, organic carbon, porewater dissolve organic carbon (DOC), and sediment pH were monitored in both sediment types at 4, 7, and 17 wks.

2-2 Hypothesis: Macroinvertebrate community toxicity will be attenuated with higher AVS and OC sediments, and with time.

3-0 MATERIALS & METHODS

3-1 Study Site

This study was conducted in a flow-through streamside mesocosm near Fairborn, OH, USA. The mesocosm was supplied with water from Warden Ditch, a relatively undisturbed headwater stream consisting of fine-grained sediments high in AVS and total organic carbon (TOC). Background stream Ni levels were 2.8 ± 0.2 µg/L, hardness 380 ± 16 mg/L of $CaCO_3$, and DOC was 3.2 ± 0.3 mg/L. The experiment began on July 31, 2007 and ended on 27-Nov-07, 2007. A full description of the streamside mesocosms is included in section 3-2.

3-2 Streamside Mesocosm Design

The streamside mesocosm was constructed from plywood and composite PVC board (Neverrot Board). The mesocosm had dimensions of 2.7 m Length x 1.4 m Width x 0.43 m Height, and was divided into 4 channels (2.7 m Length x 0.35 m Width x 0.43

m Height) in parallel. Water from Warden Ditch was delivered to the mesocosm via an electrical pump that ran continuously for 17 weeks. The streamside mesocosm outflow entered a side tributary of the stream, so there was no possibility of recirculation between out flowing water and the pump inflow (Fig 2-1).

3-3 Sediment Collection and Spiking

Sediments were collected from Warden Ditch (WD) and the Mad River (MR) and kept at 4°C until needed for Ni spiking. The WD sediments were anoxic, with high AVS and TOC. The MR sediments were predominantly sand and gravel with low AVS and TOC. Sediments were spiked with $NiCl_2 \cdot 6H_2O$ (Fisher Scientific, Pennsylvania, USA) in a serial dilution series (5 concentrations plus a reference) based on dry sediment weights. Sediment moisture content was calculated, and Ni was added to wet sediment based on dry weight calculations. Sediment dry weight was determined by weighing 10 mg of wet sediment and drying at 100°C for 24 h. All sediments, depending on volume, were mixed in buckets or spun on a mechanized rolling pin in 4 L acid cleaned containers. Ni was introduced to sediments with small volumes of water being careful to not over-saturate the sediments. Head space in the buckets/containers was purged of oxygen with N_2 gas for 5 min before sediments were sealed and stored. Sediments receiving Ni amendments were allowed to equilibrate for 1-3 days prior to deployment, and stored in cold storage (4±1°C) until needed. Subsamples of each sediment treatment and type were

collected after equilibration and during initial deployment (July 31, 2007) for chemical analysis.

3-4 Sediment Deployment

At the mesocosm site, sediments were loaded into mesh lined trays (25 cm L x 7.6 cm W x 6.3 cm H) and deployed on 1-Aug-07. Eight replicate trays were assigned to each sediment treatment, resulting in a total of 96 trays for the study (6 treatments x 2 sediment types x 8 replicates/treatment). Each channel received 24 trays of a single sediment type, arranged so that sediment Ni concentrations increased from upstream to downstream. Two channels received MR sediments, two channels received WD sediments, and both channels containing the same sediment type were identical with regard to the positioning of the trays.

3-5 Benthic Invertebrate Collection and Deployment

On 2-Aug-07, benthic invertebrates were collected from a number of nearby streams within Southwest Ohio, USA (Big Beavercreek, Little Sugar Creek, Mad River, Greenville Creek, Stillwater River, and Great Miami River). Invertebrates were collected using D-frame dipnets, Surber samplers, and kick seines. All benthic samples were pooled into 5 gallon buckets equipped with portable air pumps, and driven to the streamside mesocosm site on the day of collection.

At the study site, invertebrates were removed from buckets and distributed evenly into 24 1 L containers. A total of four day zero benthic replicates were chosen on 2-Aug-08 for QA/QC purposes. These samples were preserved in 90% ethanol in the field, and sent to Great Lakes Environmental Corporation (Traverse City, Mi) for benthos identification to family level. The pump was then turned off to lower the water level within the channels to facilitate the introduction of the invertebrates from the 1 L containers onto the sediment trays. After water was drawn down to below the tops of the trays, dividers were placed between each treatment to reduce the movement of invertebrates between trays of different treatments. Once the dividers were in place the contents of each bowl were emptied onto the respective sediment type/treatment. Following the introduction of invertebrates, flow to the channels was restored and dividers were removed.

3-6 Sediment Sampling

Sampling of the sediments was performed on 30-Aug-07, (4 wk), 20-Sept-07 (7 wk), and 27-Nov-07 (17 wk). Within a week of the initial (1-Aug-07) deployment, it was observed that sediment trays were being colonized by mats of the blue green-algae *Oscillatoria princeps*, reaching thicknesses of several millimeters by the end of the study. The purpose of the 27-Nov-0727-Nov-07 sampling date was to independently quantify the chemistry and invertebrate colonization of the upper periphyton sublayers and the lower sediment-only sublayers. During this sampling period, only the reference trays and

highest Ni treatment concentrations were sampled. During the 30-Aug-07 and 20-Sept-07 sampling events, all Ni treatment levels were sampled. Data from the 30-Aug-07 and 20-Sept-07 sampling events were analyzed together, and data from the 27-Nov-07sampling event were analyzed separately, due to the separation of the tray contents into two subsections.

During each sampling period, two trays were removed from each concentration/sediment type (MR and WD). During 30-Aug-07 and 20-Sept-07, trays were removed from the two inner channels. On 27-Nov-07, trays were removed from the two outer channels, as all of the trays from the inner channels had been removed. Upon removal, each tray was vertically divided so that one-third of the sediment was saved for chemical analyses, and two-thirds was saved for invertebrate enumeration and identification. Different chemical analyses were performed for each of the two replicate trays: AVS, SEM_{Ni}, total Ni, Fe, and Mn were measured from one replicate, and % solids and TOC were measured from the other replicate. All invertebrate samples were placed in 1 L bottles and preserved with 90% ethanol (ETOH) in the field. All chemistry samples were placed on ice in the field and returned to the laboratory, where AVS, SEM_{Ni}, and total metal samples were frozen, and % solids and TOC samples were stored at 4°C until chemical analyses were performed.

All sediment chemistry analyses were performed by Alloway Labs in Lima, OH, USA, and sediment chemical concentrations are presented as concentration on a dry

weight basis. All invertebrates were sorted and identified by Great Lakes Environmental Center (GLEC) MI, USA to the lowest practical level for non-insects (order or family), while chironomids and insects were identified to family level. Invertebrate samples were sorted using 10x dissecting microscopes, and no subsampling was required for these samples.

3-7 Porewater Dissolved Organic Carbon, Diffusive Gradient In-Thin Films and Physico-chemical monitoring

During the streamside exposure, porewater, dissolved organic carbon (DOC), and sediment (surficial and porewater) pH measurements were taken. The sediment porewater DOC samples were collected using a small aquarium air stone equipped with a tubing and syringe. The air stone was buried in the sediment of one tray/treatment and the syringe was used to extract the porewater. Porewater and DOC samples were filtered (0.45 μm) and preserved with H_2SO_4 to a pH of 2 and stored in opaque containers.

On 30-Aug-07 and 20-Sept-07, these measurements were taken within trays in the two outer channels corresponding to those trays removed on that particular date from the inner channels. The two channels with MR and WD sediments, respectively, were identical and it was assumed that porewater chemistry was identical between the two channels. This was done to minimize disturbance to the sediments removed for chemical

and invertebrate analysis, so as to minimize disruption of the Redox conditions of the sediment chemistry as well as to minimize dislodgement of colonizing invertebrates.

On 27-Nov-07 Diffusive Gradient in Thin-Films (DGTs) were used to determine Ni flux from sediments. One DGT was deployed within each sediment type for all treatments, for a total of 12 DGTs per deployment. Each DGT exposure lasted 24 h and DGTs were deployed on 9-Aug-07, 23-Aug-07, and 19-Sept-07. The DGTs were inserted to the bottom of each tray (6 cm depth), and prior to retrieval, the sediment-water interface for each DGT was carefully marked.

Upon returning to the laboratory, three 2 cm sections were removed from each DGT to assess any differences in Ni flux along a vertical gradient. The three sections that were removed corresponded to the 2 cm above the sediment water interface, the 2-4 cm depth within the sediment, and the 4-6 cm depth within the sediment. The DGT effective concentrations (C_{DGT} - µg/L) and fluxes (µg/cm^2/day) were calculated and reported for each of the three gel layers. Effective concentration is a measure of the concentration of a labile metal, in this case Ni, that comes from both diffusion in solution and release from the solid phase of the sediments, and has been shown to correlate very well with uptake by the biota (Zhang et al. 2001). Flux is the total amount of Ni that diffuses into a given area of a DGT probe over a given time period, and is correlated to the effective concentration.

During each sampling event, temperature, dissolved oxygen (DO), and conductivity were measured with a Yellow Springs Instruments (YSI) YSI-85 meter. The sediment pH measurements were taken with an YSI pH 100 meter equipped with a piercing tip probe. All values were recorded as mean ± 1 standard deviation. The upper layer (surficial) (<1 cm) and lower layer (porewater) (>5 cm) were sampled in the reference and high concentration of each sediment type periodically.

3-8 In situ Toxicity Testing

All *in situ* toxicity testing used chambers adapted from after Burton et al. (2005) with 250 μm nylon mesh windows to facilitate water and sediment exchange. Three replicate chambers were tested on each sediment type, and three additional replicate chambers were tested in the water column (inflow and outflow of each sediment type), to determine if inflow or outflow water was acutely toxic. Species used during the *in situ* test were *H. azteca, C. dilutus, Isonychia spp., P. herricki,* and *T. tubifex.* The *in situ* toxicity testing was performed on 9-Aug-07, 16-Aug-07, and 31-Aug-07 for 96 h, and each chamber received 10 organisms. At times it was necessary to reduce the number of indigenous organisms (*Isonychia spp.* and *P. herricki*) per replicate when insufficient numbers of insects were collected in the field.

3-9 Data Analysis

Since two separate trays were required to measure all of the sediment chemistry endpoints, no experimental replicate sediment chemistry samples were collected, but

analytical replicates were performed. *In situ* survival data were analyzed using one-way ANOVAs followed by Tukey's pairwise comparisons to determine differences between individual treatments. Normality of the residuals and Levene's test for equal variance were performed to determine if the data were normally distributed. If normality was violated, then a Kruskal-Wallis nonparametric test was performed. Significance was defined by p-value $\leq \alpha = 0.05$. All survival data are presented as mean % survival ± standard deviation. All physical-chemical data are reported as mean ± standard deviation when replicated data were available.

Relationships between invertebrates and sediment chemistry parameters in the 2007 streamside mesocosm study were investigated using a combination of conventional univariate parametric analysis. When sediment chemistry parameters (SEM_{Ni}, AVS, OC) were below detection limits, the value of the method detection limit (MDL) was used for these analyses (Burton and Pitt 2002). For AVS and OC, this resulted in the lowest possible value of SEM_{Ni}/AVS and (SEM_{Ni}-AVS)/f_{OC} for a given non-detect level. For the conventional analyses, relationships between the most numerically dominant benthic invertebrates densities/indices and selected sediment chemistry data (i.e. total sediment nickel, and sediment nickel bioavailability), respectively, were analyzed using a multiple linear regression procedure. For visualization purposes, a series of simple linear regressions were performed between invertebrate densities/indices and statistically significant sediment chemistry predictors for all significant covariates. Prior to these

analyses, all benthic invertebrate total abundance counts were converted to benthic densities (# individuals/m^2), based on the surface area (25 cm X 7.6 cm) of the colonization trays that were sampled (2/3 tray to benthos, 0.013m^2). Then natural log (x+1) transformed so that these data satisfied the requirements of the parametric analysis. Similarly, total Ni concentrations were also natural log transformed prior to the analysis.

A non parametric Kruskal-Wallace comparison of means test was used for bioavailability threshold determinations due to the unequal sample sizes between groups (total invertebrates, total taxa, diversity, etc.). All univariate statistical analyses were performed using version 2.7.2 of the statistical package R (R Development Core Team 2008).

Finally, EC_{10} and EC_{50} estimates and 95% confidence intervals of total nickel sediment concentrations were calculated with regard to densities of total invertebrates, Chironomidae, Elmidae, Crangonyctidae, Hyalellidae, as well as total taxa. The EC_{10} and EC_{50} estimates and confidence intervals were calculated for both sediment types independently and in combination. Similarly, EC_{10} and EC_{50} estimates and confidence intervals of all sediment type combinations were calculated for SEM_{Ni}/AVS and (SEM_{Ni}-AVS)/f_{OC} with regard to densities of total invertebrates and Chironomidae. These estimates were calculated through an iterative curve fitting spreadsheet written for Excel utilizing the "Solver" add-on function (Barnes et al. 2003). The procedure uses a sigmoidal logistic curve of the form:

Equation 1: $\frac{k}{1+\exp\left(-m\left(\frac{\log x - \log EC_{50}}{EC_{50}}\right)\right)}$,

where k equals the predicted response at concentration 0, m equals the slope of the logistic curve, and x equals the concentration of the predictor variable (total Ni, SEM_{Ni}/AVS, or (SEM_{Ni}-AVS)/f_{OC}).

4-0 RESULTS AND DISCUSSION

4-1 Sediment Chemistry and Bioavailability

Sediment chemistry was characterized, and at the beginning of the experiment (31-Jul-07) AVS concentrations differed markedly between the two sediment types. For the reference (no Ni added) sediments, AVS concentration in WD sediment was (2540 mg/kg, 74.5 µmol/g), and AVS in the MR sediment was (2.25 mg/kg, 0.07 µmol/g) (Table 2-1a.). Initial AVS concentrations in the MR sediments were similar across treatments (1.37-2.25 mg/kg, 0.04-0.07 µmol/g), and remained relatively similar during both the 30-Aug-07 and 20-Sept-07sampling events (Table 2-1b.). In contrast, AVS tended to decrease within the WD sediments between 31-Jul-07 and 20-Sept-07. For example, in the WD reference sediments, AVS concentrations decreased from 74.5 µmol/g on 31-Jul-07, to 39.9 µmol/g on 30-Aug-07, to 30.8 µmol/g on 20-Sept-07 (Table 2-1a.). This decrease in AVS from summer to early autumn is consistent with results of

previous studies, which have observed AVS to be correlated to overlying water temperature and sediment depth (Leonard et al. 1993).

Within a sediment type, AVS is similar across Ni treatment levels in MR sediment, while AVS is inversely related to SEM_{Ni} in WD sediments (Table 2-1a-b). The inverse relationship between AVS and spiked metal concentrations is consistent with previous studies, and was first reported by Di Toro et al. (1990). The lack of a corresponding decrease in AVS of MR sediments following spiking is most likely a function of the very low levels of AVS within that sediment type.

Previous empirical studies have determined that the onset of chronic metal toxicity begins at when the ratio of SEM/AVS > 8 μmol/g, and when OC normalized excess SEM is greater than 100-150 μmol/g. These results have been confirmed in subsequent field validations of the SEM/AVS OC model of metal toxicity (Burton et al. 2005; Di Toro et al. 2005; Burton 2010; Nguyen et al. 2011). Using the SEM/AVS criteria for the WD sediments, we would predict toxicity at the highest Ni concentration for all sampling dates, but would predict the possibility of toxicity at the second highest Ni treatment level (Table 2-1a.). In contrast, toxicity of MR sediments would be predicted for all non reference treatments; as both SEM_{Ni}/AVS and OC normalized excess SEM_{Ni} exceed predicted chronic toxicity thresholds (Table 2-1b.). However, MR SEM_{Ni} concentrations are low, and in the majority of cases, SEM_{Ni}-AVS < 2 (Table 2-1b.). Therefore, despite the high values of SEM_{Ni}/AVS and SEM_{Ni}-AVS/foc of non-

reference MR sediments, because SEM_{Ni}-AVS < 2 μmol/g for many MR treatments, chronic toxicity of MR sediments might not be apparent. It should be noted, however, that the low levels of SEM_{Ni} for the spiked MR sediments are a function of low levels of AVS, so this criteria is probably not as predictive as SEM_{Ni}/AVS or (SEM_{Ni}-AVS)/foc for these sediments.

Total Ni concentrations were followed through time, and were showing that Ni concentrations decreased in both sediment types (Table 2-1a - 2-1c). The magnitude of Ni efflux was positively correlated to the initial Ni spiking concentration for both sediment types. Overall, Ni efflux from the sediments was greater in the MR sediments, which would be expected given the low levels of AVS and OC (Table 2-1c). Ni was lost from all of the WD sediments, even for those treatments where AVS exceeded SEM_{Ni} (Table 2-1c). Metal efflux from spiked sediments, even when all of the metal is theoretically bound to AVS, has been observed in several studies (Boothman et al. 2001; Naylor et al. 2006). Metals within porewater are in a "pseudo steady state", and the release of metals into the overlying water can occur as a result of reductive dissolution of manganese oxides, particularly in the case of Ni, which is the most soluble of the divalent metals (Naylor et al. 2006).

4-2 Sediment Porewater pH and Temperature

Sediment pH was higher overall in the MR reference sediments than in the WD sediments. Furthermore, MR reference sediment was similar for surficial (<2 cm) and

deep (>3 cm) sediment, while pH for WD reference sediments decreased with depth (Table 2-3). Relative to the reference trays, sediment pH was lower for the highest Ni treatment concentrations for both sediment types, and sediment pH decreased with depth for both MR and WD sediments (Table 2-3). The decrease in sediment pH with depth in the high Ni treatment for all sediments suggests that metal concentrations are likely higher in the deeper sediments of the colonization trays relative to the surficial sediments (Di Toro et al. 2005). Overall, sediment temperatures were similar across sediment types and treatments (22.0-22.5˚C) (Table 2-3), which is not unexpected given that the relatively small colonization trays were exposed on all sides with surface water.

4-3 Porewater Dissolved Organic Carbon and TOC

Porewater DOC samples from both sediment types were collected on 30-Aug-07, 2007 and 20-Sept-07, 2007. On 30-Aug-07, porewater DOC was below detection limit (5.0 mg/L) for all samples, with the exception of the WD reference tray (5.2 mg/L). A more sensitive analytical method was used with a lower detection limit for on 20-Sept-07. For both sediment types, the highest DOC concentrations were in the reference treatments, and the relative decline between reference DOC and DOC of trays with Ni spiked sediments was highest in the MR sediments.

Since DOC is able to mitigate metal toxicity by complexation with metals it would be expected that relatively more metals would be bound to DOC in the MR

sediments, which have much lower binding capacity than the WD sediments (Doig and Liber 2006). However, the overall effect on sediment Ni availability is minor at DOC concentrations between 5-10 mg/L, which is typical of the values observed in this study (Doig and Liber 2006).

Finally, TOC concentrations differed between MR and WD sediments. The large-grained erosional MR sediments contained very little TOC, and in most instances, TOC concentrations were below detection limits (1000 mg/kg, or 0.1%) (Table 2-1b). In contrast, TOC for the WD sediments was relatively high, and averaged between 5%-8% for most of the colonization trays (Table 2-1a).

In addition to AVS, organic carbon will also bind free metals and the relatively high proportions of TOC in the WD sediments suggests that they will have additional metal binding capacity in excess of that provided by AVS (Mahony 1996; Di Toro et al. 2005; Burton 2010).

4-4 Diffusive Gradient in-Thin Films

Both effective concentrations (C_{DGT}, in µg/L) of Ni and flux of Ni ($\mu g \cdot cm^{-2} \cdot day^{-1}$) into the DGT probes were higher in WD sediments (Table 4a.) than in MR sediments (Table 2-4b.). This result can be explained by the higher Ni spiking levels in the WD sediment treatments. Also, the amount C_{DGT} (Ni concentration at DGT surface with respect to time) was found inversely related to AVS and TOC content (Costello et

al. 2012). Costello et al. (2012) found that C_{DGT} values were much higher in MR sediments, and this may have been due to porosity differences between the MR and WD sediments. Although overall binding capacity was much higher in WD sediments, porewater metal exists in a dynamic "pseudo-steady state" that varies as a function of physical hydrologic perturbations as well as dissolution of metals bound to Fe and Mn oxyhydroxides (Naylor et al. 2006).

In both sediment types, C_{DGT} and flux increased with initial Ni spiking concentrations and decreased over time (Costello et al. 2012). This is consistent with the overall loss of total sediment Ni in this study (Tables 2-1a. - 2-1c), and other field exposures with spiked sediments (Boothman et al. 2001, Costello et al. 2012). Both C_{DGT} and flux were higher within the sediments, compared to the bottom 2cm of the surface water, and in general, Ni C_{DGT} and fluxes were higher in the 4-6cm deep sediments than the 2-4cm deep sediments (Tables 2-4a.- 2-4b). Costello et al. (2012) found that C_{DGT} concentrations were higher in deep sediments (> 2cm), and they attributed the flux in the top sediment layers (< 2 cm) to physical changes when inserting the DGT probes. The increase in both C_{DGT} and flux with increasing depth throughout the field exposure suggests that Ni was preferentially retained in the deeper sediment layers of the colonization trays, which is consistent with other similar experimental designs (Boothman et al. 2001, Nguyen et al. 2011).

4-5 In Situ Toxicity Testing

During the three *in situ* toxicity tests there were no indications of acute Ni effects to caged organisms placed on the spiked sediments. However, when testing the reference versus the highest concentration in the first run (9-Aug-07) there was a significantly fewer *C. dilutus* (*p*-value =0.037) and marginally significantly fewer (p-value =0.067) *P. herricki*.

During the *in situ* tests it became apparent that an additional stressor was likely compromising the reference survival of *C. dilutus*. One possibility was the periphyton layer that had formed on the surface of the sediments in the streamside mesocosm, and which was dominated by *Oscillatoria princeps* (blue-green algae), a species known to produce toxins. To test this hypothesis, a comparison test with *C. dilutus* was performed within the mesocosm on the reference sediments (MR) and *in situ* within the MR. There was a marginally significant difference (*p*-value = 0.08) between the streamside reference treatment and MR exposures, suggesting possible stress from the periphyton.

During the three *in situ* tests conducted within the mesocosm there were only two significant or marginally significant responses (*C. dilutus* and *P. herricki*). In a similar 7 day laboratory Ni sediment flow thru study, *P. herricki* did not respond (> 90% survival in highest treatment) to even very high concentrations of Ni (~6000 mg/kg) (Chapter 4), which would suggest the presence of acute Ni toxicity was highly unlikely for any of the three *in situ* tests.

4-6 Biological Responses

Benthic community differences were examined in response to increasing sediment Ni. The benthic densities of the four invertebrate taxa (Chironomidae, Crangonyctidae, Hyalellidae, and Elmidae), total invertebrates, and total taxa decreased as sediment Ni concentrations increased (Tables 2-5, 2-6). Chironomidae and Crangonyctidae responses to total Ni sediment concentrations were also affected by both sediment type and by sampling date, while Hyalellidae and Elmidae responses to total Ni sediment concentrations were affected by sediment type (Table 2-6). For all taxa, benthic densities were higher in the low AVS MR sediment, but densities decreased with increasing sediment Ni concentrations (Figs. 2-2 – 2-6). Furthermore, the slopes of the negative relationships between benthic densities and Ni concentrations were generally steeper in the high AVS WD sediments.

The effect of sampling date appears to be taxa specific. Chironomid densities were higher in August than in September, but Crangonyctidae densities were higher in September than August. Both Hyalellidae and Elmidae were similar across sampling dates. Total taxa were higher in September than in August, and declined with increasing Ni concentration on both dates (Fig. 2-7).

Benthic densities of Chironomidae, Crangonyctidae, and Hyalellidae decreased with increasing SEM_{Ni}/AVS (Figs. 2-2 – 2-5). Taxa specific relationships between sediment type and SEM_{Ni}/AVS were generally similar to those between sediment type and total Ni concentrations. Chironomid and Crangonyctidae densities were overall

higher in the MR sediments, and declined with increasing SEM_{Ni}/AVS values. Hyalellidae, Chironomidae, Crangonyctidae densities and total invertebrates were higher in MR sediments than in WD sediments, and declined with increasing SEM_{Ni}/AVS in both sediment types (Figs. 2-2 – 2-6).

Benthic densities of Chironomidae, Crangonyctidae, Hyalellidae, total invertebrates, total taxa, and Shannon-Wiener diversity decreased with increasing SEM_{Ni}-AVS/foc (Table 2-7). In addition, the negative relationship between SEM_{Ni}-AVS/foc and Hyalellidae and total invertebrate densities was more pronounced in the WD sediments (Fig. 2-8). Total invertebrate taxa were higher overall in MR sediment, and were higher in September than in August (Fig. 2-9). Shannon-Wiener diversity declined with increasing SEM_{Ni}-AVS/foc, particularly in August, and was higher overall in September than in August (Fig. 2-9).

When overlying periphyton and underlying sediments were analyzed separately, both chironomid densities and total benthic densities were significantly related to sediment type, substrate, and Ni treatment level. In particular, colonizing invertebrates exhibited a strong preference for the upper periphyton layers of both sediment types, and were most abundant overall in the periphyton layers covering the high nickel treatment trays for both sediment types (Fig. 2-10). These patterns persisted even though concentrations of total Ni, levels of SEM_{Ni}/AVS, and levels of SEM_{Ni}-AVS/foc were higher in those trays (Fig. 2-10). It is worth noting that the upper periphyton layers also

had the highest concentrations of total Mn measured during the colonization study, although it is not clear as to whether the Mn in these samples provided any protective effect.

The increased abundances of colonizing invertebrates in the upper periphyton layers could be the result of a preference for periphyton vs. sediment. However, it could have been from avoidance of higher concentrations of Ni below the periphyton, or simply a preference for colonizing the substratum: surface water interface. Many chironomid species, the dominant taxa in this study, exhibit a strong preference for inhabiting dense filamentous periphyton mats which provide both protection and food, even those which contain cyanobacteria (Sabater and Munoz 2000). Furthermore, total Ni concentrations in the periphyton layer of the high Ni treatment trays were lower compared to the underlying sediment for both sediment types (Fig. 2-10). Finally, most invertebrates in this study were epibenthic, and their higher numbers in the upper periphyton layers could simply be a consequence of their preference for living at the substratum: surface water interface. What is less clear is why these invertebrates were even more abundant in the periphyton mats covering the high Ni treatment trays, compared to the periphyton mates covering control treatment trays.

The significant negative correlations to levels of total nickel and bioavailable nickel, in general, persisted across all numerically dominant taxa, as well as the total number of invertebrates and total invertebrate taxa. Overall, invertebrate responses to the

effects of total sediment nickel concentrations were similar to the effects of SEM_{Ni}/AVS, as well as organic carbon normalized SEM_{Ni}-AVS. In large part, these similar responses were the result of the high degrees of correlation between total Ni, SEM_{Ni}/AVS, and SEM_{Ni}-AVS/foc within a particular sediment type (Table 2-7). The overall ability of the high AVS – high OC WD sediment to theoretically mitigate potential metal toxicity is evidenced by the overall lower ranges of SEM_{Ni}/AVS and SEM_{Ni}-AVS/foc across the range of nickel treatment levels. Although the general pattern of decreasing invertebrate abundance was similar for increasing total Ni and bioavailable Ni. The overall lower significance values for the effects of bioavailable nickel (Tables 2-6 and 2-8), compared to the effects of total nickel (Table 2-7), suggest that bioavailable nickel may be better at predicting invertebrate densities than total nickel. Furthermore, Shannon-Wiener diversity was only significantly related to SEM_{Ni}-AVS/foc, thus suggesting the OC normalized SEM to be the best predictor of sediment metal bioavailability (i.e. Di Toro et al. 2005).

Overall, these results can be explained by multiple linear models. However, previous studies have found significant invertebrate impacts only after particular bioavailability thresholds have been exceeded (Di Toro et al. 2005; Burton et al. 2005; Nguyen et al. 2011). One possible explanation could be the different experimental designs used in these experiments. In previous field colonization experiments, only 2-3 treatments plus a control were used, and as a result those studies were limited to ANOVA

analysis. By using six treatment levels, we were able to perform regression analysis and therefore determine functional relationships across the range of experimental treatments, as well as to determine whether there is an overall treatment effect (slope ≠0). With the exception of the reference trays, all of the treatment levels of the MR sediments exceeded predicted bioavailability thresholds, so one would expect a decline in invertebrate responses across the entire concentration gradient, and in general, that was the result. However, for the WD sediments, three of the Ni treatments plus the reference were predicted below the SEM/AVS thresholds, yet even in that "negative" range, there was still an observable overall negative relationship between invertebrate responses. This probably reflects the dynamic relationship between porewater Ni and sediment as some of the Ni is more loosely bound to Mn and Fe oxyhydroxides (Naylor et al. 2006). The relationships for SEM_{Ni}/AVS are harder to visualize because ratios where no toxicity was predicted were very close to 1.

In addition to examining the functional relationships between invertebrates and total and bioavailable nickel, the presence of invertebrate responses above and below previously observed bioavailability thresholds (SEM-AVS ≤ 0 and SEM/AVS ≤ 1 SEM_{Ni}-AVS/foc < 130) were examined (USEPA 2005). Field validations of the model further refined these predictions, finding no zinc toxicity when SEM/AVS < 2, and OC normalized excess SEM was below 150 (Burton et al. 2005). In this study, two of the MR treatment levels (20-Aug-07) exceed previously observed bioavailability thresholds

for the Caenidae responses. Respectively, these MR treatment levels were less than: 13.02 µmol/g (SEM_{Ni}-AVS), 230.9 µmol/g (SEM_{Ni}/AVS), and 13016 µmol/g SEM_{Ni}-AVS/foc however, these were not statistically significant ($p = 0.081$). Caenidae responses were the only invertebrate responses that showed a marginal no effect levels using the Dunnett's test. In contrast, the remaining treatment levels and the reference sediments fall below predicted and observed thresholds.

In many instances, the effects of sediment porewater nickel, or nickel bioavailability are only apparent after accounting for sediment type. In this experiment, channels containing MR sediment were in parallel to channels containing WD sediment, so the likelihood of a particular organism colonizing either sediment type was equal. Further, pre-seeding of all trays with invertebrates was similar across sediment and nickel treatment levels. The higher invertebrate densities on the low-AVS MR sediment likely reflect a general habitat preference for gravel/sandy substrate for the taxa used to seed the experiment, which were collected primarily from streams with gravel/sandy substrata. This is especially true for EPT taxa, and other aquatic insect taxa. Furthermore, although the ranges of both SEM_{Ni}/AVS and (SEM_{Ni}-AVS)/f_{OC} are more narrow in WD sediment than in MR sediment, the negative slopes of the relationships between invertebrate densities and bioavailable nickel are steeper in the WD sediments, further suggesting that this sediment is less optimal than the MR sediment. In previous field colonization studies (i.e. Burton et al. 2005; Nguyen et al. 2011, Costello et al. 2011), spiked sediments were

returned to their original sites, and were colonized by organisms that were already living in those systems. This may explain why the sediment type effect was so pronounced in this mesocosm study, relative to prior similar studies conducted *in situ*.

During the study, surface water was drawn from WD to feed the channels, and there is evidence that some invertebrates from WD survived passage through the pump unscathed to colonize the trays. Shannon-Wiener diversity increased significantly from 0.57 in August to 1.08 in September ($p < 0.001$). Similarly, ecological evenness increased significantly between August (0.34) and September (0.55) ($p < 0.001$). These results indicate more invertebrate taxa were colonizing trays in September, and that numbers of individuals for across taxa were more evenly distributed in September than in August, as organisms that were initially seeded drifted from the mesocosm and increasing numbers of organisms from WD colonized the trays. Although this experimental artifact may have been responsible for the significant sediment type effect, there was still a general pattern of negative relationships between invertebrates and total and bioavailable nickel for both sediment types, which support the (SEM-AVS)/foc model of metal bioavailability.

Finally, EC_{10} and EC_{50} estimates were calculated for the majority of the invertebrate endpoints analyzed in this study and total Ni, SEM_{Ni}/AVS and (SEM_{Ni}-AVS)/f_{OC}, both separately by sediment type and combined (Table 2-9). Both EC_{10} and EC_{50} for all invertebrate endpoints were lower in MR sediments than WD sediments,

which would be expected given the higher binding capacity for Ni in the WD sediments. However, EC_{10} and EC_{50} for total invertebrates and Chironomids in response to SEM_{Ni}/AVS and (SEM_{Ni}-AVS)/foc were lower in MR sediments than in WD sediments, which reinforces the notion that WD sediments are relatively suboptimal for the organisms in this experiment (Table 2-9). In most cases, confidence intervals were large, particularly for EC_{10} values, although this is probably the result of the lack of data surrounding the EC_{10} in most instances (Barnes et al. 2003).

5-0 GENERAL CONCLUSIONS

The objectives of this study were to investigate the relationships between Ni sediment concentration, bioavailability, and toxicity to aquatic macroinvertebrates in a streamside flow-through mesocosm. Sediment Ni toxicity was determined by transplanting macroinvertebrate communities and exposing *Hyalella azteca, Chironomus dilutus, Tubifex tubifex*, *Isonychia* spp., and *Psephenus herricki* in the mesocosm system. Changes in AVS, total Ni, SEM-Ni, total Fe, total Mn, organic carbon, porewater dissolve organic carbon (DOC), and sediment pH were monitored in both sediment types at 4, 7, and 17 wks. These objectives were met as evidenced by showing benthic responses to bioavailable Ni, demonstrating the relationships between Ni bioavailability and sediment type, as well as binding to AVS and organic carbon, and also benthic organisms showed higher densities on MR sediments over WD sediments.

Both AVS and OC appeared to mitigate Ni toxicity, as evidenced by the significant negative relationships between invertebrate densities and both SEM_{Ni}/AVS and (SEM_{Ni}-AVS)/*foc*. However, results of this study also demonstrated that the (SEM_{Ni}-AVS)/*foc* model of metal bioavailability alone does not fully explain the observed results. Benthic invertebrates in this experiment demonstrated a pronounced preference for colonizing the sandy/gravel, low AVS MR sediments, even though overall Ni bioavailability, as predicted by the (SEM-AVS)/*foc* model, were generally higher in those sediments than in the WD sediments. Finally, when periphyton levels were high, as in the November 2007 samples, invertebrates demonstrated a strong preference for colonizing periphyton, regardless of whether the periphyton covered the sandy/gravel sediment or high clay/silt sediment.

The hypothesis was supported in which the benthic communities demonstrated negative relationships to increasing Ni and SEM/AVS values. The mesocosm allowed for examination of Ni field effects using natural water and natural conditions through time. Macroinvertebrate communities were also showing sediment type differences, and these were prevailing through time. These results are showing how benthic macroinvertebrate community structure changed with increasing Ni, and with time. These results are important for understanding contaminated sediment concerns, and can also show how trophic levels could be negatively affected by changes in benthic community structure.

Table 2-1a. Warden Ditch sediment chemistry parameters.

Ni Treatment Level	Date	SEM_{Ni}/AVS (µmol/g)	SEM_{Ni}-AVS/*f*OC (µmol/g)	SEM_{Ni}-AVS (µmol/g)	Total Ni (mg/kg)	Total Ni (µmol/g)	SEM_{Ni} (µmol/g)	AVS (µmol/g)	Total Mn (mg/kg)	Total Fe (mg/kg)	TOC (%)
WD Reference	31-Jul-07	0.001	-1375.5	-74.4	18	0.30	0.1	74.52	411	20800	5.4
WD 658	31-Jul-07	0.126	-848.7	-45.9	658	11.17	6.6	52.52	428	21200	5.4
WD 1290	31-Jul-07	0.329	-578.2	-31.7	1290	21.90	15.6	47.24	378	20400	5.5
WD 2240	31-Jul-07	0.507	-426.2	-25.7	2240	38.03	26.5	52.23	365	20800	6.0
WD 5180	31-Jul-07	4.807	1108.4	57.4	5180	87.95	72.5	15.08	369	19900	5.2
WD 9380	31-Jul-07	7.934	2027.1	111.3	9380	159.25	127.3	16.05	414	20600	5.5
WD Reference	30-Aug-07	0.011	-640.4	-39.5	16	0.28	0.5	39.90	388	18400	6.2
WD 658	30-Aug-07	0.252	-270.3	-18.4	574	9.75	6.2	24.56	414	20200	6.8
WD 1290	30-Aug-07	0.138	-1080.3	-76.6	1030	17.49	12.3	88.90	414	20100	7.1
WD 2240	30-Aug-07	0.317	-543.3	-41.7	1750	29.71	19.4	61.03	376	18800	7.7
WD 5180	30-Aug-07	3.543	574.5	35.2	4340	73.68	49.1	13.85	399	20800	6.1
WD 9380	30-Aug-07	9.048	1235.2	85.5	8340	141.60	96.1	10.62	408	21400	6.9
WD Reference	20-Sep-07	0.005	-604.4	-30.6	10	0.17	0.2	30.81	421	13600	5.1
WD 658	20-Sep-07	0.254	-238.5	-18.2	387	6.57	6.2	24.35	409	16700	7.6
WD 1290	20-Sep-07	0.150	-756.2	-49.6	774	13.14	8.8	58.39	359	16800	6.6
WD 2240	20-Sep-07	0.540	-226.7	-14.2	1660	28.18	16.6	30.81	330	16300	6.3
WD 5180	20-Sep-07	1.957	334.5	19.1	2720	46.18	39.0	19.95	336	17200	5.7
WD 9380	20-Sep-07	23.248	2137.5	111.6	5540	94.06	116.6	5.02	339	17000	5.2

Table 2-1b. Mad River sediment chemistry parameters.

Ni Treatment Level	Date	SEM_{Ni}/AVS (µmol/g)	SEM_{Ni}-AVS/*f*OC (µmol/g)	SEM_{Ni} - AVS (µmol/g)	Total Ni (mg/kg)	Total Ni (µmol/g)	SEM_{Ni} (µmol/g)	AVS (µmol/g)	Total Mn (mg/kg)	Total Fe (mg/kg)	TOC (%)
MR Reference	31-Jul-07	0.697	-20.02	-0.02	17	0.28	0.05	0.07	300	14700	<0.1*
MR 80	31-Jul-07	37.719	1475.93	1.48	80	1.35	1.5	0.04	639	5670	<0.1*
MR 140	31-Jul-07	60.311	2471.07	2.47	140	2.38	2.5	0.04	282	5500	<0.1*
MR 278	31-Jul-07	71.672	4084.81	4.08	278	4.72	4.1	0.06	313	7420	<0.1*
MR 692	31-Jul-07	230.865	13016.36	13.02	692	11.75	13.1	0.06	275	5270	<0.1*
MR 1030	31-Jul-07	223.238	13301.75	13.30	1030	17.49	13.4	0.06	409	8040	<0.1*
MR Reference	30-Aug-07	0.83	-5.00	-0.01	9	0.15	0.03	<0.03*	356	6810	<0.1*
MR 80	30-Aug-07	21.79	623.65	0.62	135	2.29	0.7	<0.03*	374	7930	<0.1*
MR 140	30-Aug-07	37.97	1109.22	1.11	89	1.51	1.1	<0.03*	373	4670	<0.1*
MR 278	30-Aug-07	56.03	1650.81	1.65	155	2.63	1.7	<0.03*	339	8200	<0.1*
MR 692	30-Aug-07	32.67	1942.07	1.94	112	1.90	2.0	0.06	319	4530	<0.1*
MR 1030	30-Aug-07	35.69	1699.73	1.70	169	2.87	1.7	0.05	255	4500	<0.1*
MR Reference	20-Sep-07	0.27	-43.39	-0.04	11	0.19	<0.016*	0.06	377	9780	<0.1*
MR 80	20-Sep-07	11.57	586.31	0.59	72	1.23	0.6	0.06	480	8230	<0.1*
MR 140	20-Sep-07	18.18	358.25	0.89	103	1.75	0.9	0.05	331	6980	0.2
MR 278	20-Sep-07	30.37	1559.80	1.56	138	2.34	1.6	0.05	639	5900	<0.1*
MR 692	20-Sep-07	34.72	2127.07	2.13	126	2.14	2.2	0.06	269	5040	<0.1*
MR 1030	20-Sep-07	47.65	2327.03	2.33	151	2.56	2.38	0.05	407	7380	<0.1*

*** below detection limit**

or TOC below detection limits were calculated using minimum detection limits

Table 2-1c. Changes in sediment chemistry parameters by sampling date.

	Porewater Sediment Chemistry Measure					
Ni Treatment Level	Δ Total Ni (%) Jul 31- Aug 30	Δ Total Ni (%) Jul 31 - Sep-20	Δ SEMNi (%) Jul 31- Aug 30	Δ SEMNi (%) Jul 31 - Sep 20	Δ AVS (%) Jul 31- Aug 30	Δ AVS (%) Jul 31 - Sep 20
WD Reference	-7	-42	324	53	-46	-59
WD 658	-13	-41	-6	-6	-53	-54
WD 1290	-20	-40	-21	-44	88	24
WD 2240	-22	-26	-27	-37	17	-41
WD 5180	-16	-47	-32	-46	-8	32
WD 9380	-11	-41	-25	-8	-34	-69
MR Reference	-45	-33	-41	-68	-51	-12
MR 80	69	-9	-57	-58	-15	38
MR 140	-37	-26	-55	-62	-17	25
MR 278	-44	-50	-59	-61	-40	-8
MR 692	-89	-88	-85	-84	2	5
MR 1030	-76	-78	-87	-82	-13	-12

	Porewater Sediment Chemistry Measure (cont.)					
Ni Treatment Level	Δ Total Mn (%) Jul 31- Aug 30	Δ Total Mn (%) Jul 31 - Sep 20	Δ Total Fe (%) Jul 31- Aug 30	Δ Total Fe (%) Jul 31 - Sep 20	Δ TOC (%) Jul 31- Aug 30	Δ TOC (%) Jul 31 - Sep 20
WD Reference	-6	2	-12	-35	14	-6
WD 658	-3	-4	-5	-21	26	41
WD 1290	10	-5	-1	-18	29	20
WD 2240	3	-10	-10	-22	27	3
WD 5180	8	-9	5	-14	18	10
WD 9380	-1	-18	4	-17	26	-5
MR Reference	19	26	-54	-33	0	0
MR 80	-41	-25	40	45	0	0
MR 140	32	17	-15	27	0	0
MR 278	8	104	11	-20	0	149
MR 692	-22	-34	-44	-37	0	0
MR 1030	-7	48	-15	40	0	0

Table 2-2. Physico-chemical readings from continuous monitoring with an YSI 650 at Warden Ditch from 24-Sept-07 to 14-Oct-07.

	Temperature	Specific Conductance	Turbidity	pH	DO	Hardness
	(°C)	(mS/cm)	NTU	(units)	(mg/L)	(mg/L of CaCO3)
Mean	17.36	0.494	13.4	7.5	9.04	380.3
St.Dev	2.47	0.051	3.5	0.07	1.72	15.7
Max	21.11	0.705	33.7	7.69	13.3	398
Min	11.79	0.4	9	7.29	3.59	368

Table 2-3. Temperature and pH measurements in surficial and porewater sediments in the streamside mesocosm. Measurements were taken in the references and high (MR 1030, WD 9380) treatments between 5-Aug-07 and 24-Sept-07. Surficial sediment (SS) top layer of sediment (< 2 cm), deeper Sediment (DS) bottom layer of sediment (> 3 cm).

	MR Reference	MR Reference	MR Reference	MR Reference	MR	MR	MR	MR
					1030	1030	1030	1030
	SS	SS (Temp)	DS	DS (Temp)	SS	SS (Temp)	DS	DS (Temp)
	(pH)		(pH)		(pH)		(pH)	
Mean	7.03	22.07	7.01	22.2	6.88	21.99	6.72	22.04
St.Dev	0.14	1.34	0.16	1.38	0.18	1.35	0.2	1.37
	WD Reference	WD Reference	WD Reference	WD Reference	WD 9380	WD 9380	WD 9380	WD 9380
	SS	SS (Temp)	DS	DS(Temp)	SS	SS (Temp)	DS	DS (Temp)
	(pH)		(pH)		(pH)		(pH)	
Mean	6.47	22.13	6.36	22.52	6.5	22.01	6.35	22.18
St.Dev	0.33	1.31	0.25	1.43	0.25	1.32	0.25	1.32

Table 2-4a. Ni effective concentration (C_{DGT} - µg/L) and daily diffusive flux (µg · L^{-1} · day^{-1}) as measured by DGT probes by date, Warden Ditch sediments.

		Aug. 9		Aug. 23		Sept. 19	
Ni Treatment	**Depth**	**C_{DGT} (µg/L)**	**Ni Flux (µg·L-1·d-1)**	**C_{DGT} (µg/L)**	**Ni Flux (µg·L-1·d-1)**	**C_{DGT} (µg/L)**	**Ni Flux (µg·L-1·d-1)**
Reference	2cm surface	0.25	0.001	0.24	0.001	0.45	0.002
	2-4cm depth	0.46	0.002	2.53	0.013	0.67	0.003
	4-6cm depth	0.27	0.001	0.49	0.002	0.53	0.003
658 mg/kg	2cm surface	1.09	0.005	1.45	0.007	1.81	0.009
	2-4cm depth	5.43	0.027	12.67	0.063	17.19	0.085
	4-6cm depth	4.8	0.024	20.81	0.103	13.57	0.067
1290 mg/kg	2cm surface	35.29	0.174	22.62	0.112	16.29	0.081
	2-4cm depth	44.34	0.219	28.96	0.143	32.57	0.161
	4-6cm depth	28.96	0.143	24.43	0.121	25.34	0.125
2240 mg/kg	2cm surface	71.48	0.353	144.8	0.716	0.31	0.002
	2-4cm depth	72.39	0.358	208.1	1.029	1.36	0.007
	4-6cm depth	99.53	0.492	289.6	1.431	37.1	0.183
5180 mg/kg	2cm surface	26.24	0.13	99.5	0.492	7.15	0.035
	2-4cm depth	615.3	3.041	171.9	0.85	208.1	1.029
	4-6cm depth	1085.8	5.367	289.6	1.431	162.9	0.805
9380 mg/kg	2cm surface	1357.3	6.708	778.2	3.846	15.38	0.076
	2-4cm depth	4071.9	20.13	1538.3	7.603	83.25	0.411
	4-6cm depth	5881.6	29.07	2533.6	12.52	298.6	1.476

Table 2-4b. Ni effective concentration (C_{DGT} - µg/L) and daily diffusive flux (µg · L^{-1} · day^{-1}) as measured by DGT probes by date, Mad River sediments.

		Aug. 9		Aug. 23		Sept. 19	
Ni Treatment	**Depth**	**C_{DGT} (µg/L)**	**Ni Flux (µg·L-1·d-1)**	**C_{DGT} (µg/L)**	**Ni Flux (µg·L-1·d-1)**	**C_{DGT} (µg/L)**	**Ni Flux (µg·L-1·d-1)**
Reference	2cm surface	4.16	0.021	0.6	0.003	0.16	0.001
	2-4cm depth	1.9	0.009	1.9	0.009	0.12	0.001
	4-6cm depth	7.87	0.039	1.09	0.005	0.52	0.003
80 mg/kg	2cm surface	19.91	0.098	3.35	0.017	0.16	0.001
	2-4cm depth	99.53	0.492	17.19	0.085	1.54	0.008
	4-6cm depth	208.1	1.029	42.53	0.21	4.61	0.023
140 mg/kg	2cm surface	5.61	0.028	56.1	0.277	0.89	0.004
	2-4cm depth	27.15	0.134	99.53	0.492	14.48	0.072
	4-6cm depth	153.8	0.76	88.68	0.438	36.19	0.179
278 mg/kg	2cm surface	23.53	0.116	6.7	0.033	1.36	0.007
	2-4cm depth	108.6	0.537	29.86	0.148	15.38	0.076
	4-6cm depth	289.6	1.431	34.38	0.17	22.62	0.112
692 mg/kg	2cm surface	30.77	0.152	12.67	0.063	1.36	0.007
	2-4cm depth	47.96	0.237	38	0.188	10.86	0.054
	4-6cm depth	126.7	0.626	108.6	0.537	19.91	0.098
1030 mg/kg	2cm surface	199.1	0.984	8.42	0.042	1.45	0.007
	2-4cm depth	470.5	2.326	90.49	0.447	0.58	0.003
	4-6cm depth	515.8	2.549	75.1	0.371	6.97	0.034

Table 2-5. Final multiple linear regression models of numerically dominant invertebrate responses and total invertebrate taxa to total sediment Ni concentrations. Initial full model (Biotic response = Total Ni + Sediment Type + Date + Total Ni:Sediment Type + Total Ni:Date) reduced through iterative model reduction until a final model with all significant independent variables was obtained.

					Overall Model:			
Response	**Coefficients**	**Value**	**t**	**P(>t)**	**r^2**	**F**	**df**	**P**
Total Density	Intercept	9.78	52.6	<0.001	0.52	24.1	2,45	<0.001
	Total Ni	-0.11	-3.09	0.003				
	Sediment Type	-0.48	-3.25	0.002				
Chironomidae	Intercept	12.75	10.8	<0.001	0.51	15.5	3,44	<0.001
	Total Ni	-0.11	-2.63	0.011				
	Sediment Type	-0.52	-3.12	0.003				
	Date	-0.38	-2.81	0.007				
Hyalellidae	Intercept	8.07	12	<0.001	0.32	10.3	2,45	<0.001
	Total Ni	-0.3	-2.2	0.033				
	Sediment Type	-1.03	-1.95	0.057				
Total Taxa	Intercept	4.97	7.78	<0.001	0.32	10.6	2,45	<0.001
	Total Ni	-0.05	-2.89	0.006				
	Date	0.25	3.42	0.001				
Crangonyctidae	Intercept	-11.6	4.99	0.034	0.25	7.65	4,43	<0.001
	Total Ni	-0.79	-1.76	0.086				
	Sediment Type	-1.42	-2.3	0.026				
	Date	1.96	3.17	0.003				
	TotalNi:SedType	0.83	1.67	0.102				
Elmidae	Intercept	6.28	7.24	<0.001	0.15	3.98	2,45	0.026
	Total Ni	-0.48	-2.75	0.009				
	Sediment Type	1.46	2.12	0.039				

Table 2-6. Final multiple linear regression models of numerically dominant invertebrate responses and total invertebrate taxa to total molar SEM_{Ni}/AVS. Initial full model (Biotic response = (SEM_{Ni}/AVS) + Sediment Type + Date + (SEM_{Ni}/AVS):Sediment Type + (SEM_{Ni}/AVS):Date) reduced through iterative model reduction until a final model with all significant independent variables was obtained.

					Overall Model:			
Response	**Coefficients**	**Value**	**t**	**P(>t)**	**r^2**	**F**	**df**	**P**
Total Density	Intercept	9.6	59.1	<0.001	0.48	20.1	2,45	<0.001
	SEMNi/AVS	-0.01	-2.22	<0.001				
	Sediment Type	-1.01	-5.83	<0.001				
Chironomidae	Intercept	12.7	10.5	<0.001	0.49	14.3	3, 44	<0.001
	SEMNi/AVS	-0.01	-2.21	0.032				
	Sediment Type	-1.01	-5.58	<0.001				
	Date	-0.39	-2.81	0.007				
Crangonyctidae	Intercept	6.84	8.27	<0.001	0.20	5.63	2,45	0.007
	SEMNi/AVS	-0.06	-2.52	0.015				
	Sediment Type	-2.95	-3.34	0.002				

Table 2-7. Pearson correlation coefficients between total sediment Ni and Ni sediment bioavailability measures.

	Mad River Sediments			
	Total Ni	**SEMNi/AVS**	**SEMNi-AVS**	**(SEMNi-AVS)/*f*OC**
Total Ni	1	0.84	0.82	0.79
SEMNi/AVS	0.84	1	0.85	0.85
SEMNi-AVS	0.82	0.85	1	0.98
(SEMNi-AVS)/*f*OC	0.79	0.85	0.98	1
	Warden Ditch Sediments			
	Total Ni	**SEMNi/AVS**	**SEMNi-AVS**	**(SEMNi-AVS)/*f*OC**
Total Ni	1	0.71	0.87	0.85
SEMNi/AVS	0.71	1	0.86	0.91
SEMNi-AVS	0.87	0.86	1	0.99
(SEMNi-AVS)/*f*OC	0.85	0.91	0.99	1
	Both Sediments Combined			
	Total Ni	**SEMNi/AVS**	**SEMNi-AVS**	**(SEMNi-AVS)/*f*OC**
Total Ni	1	-0.19	0.71	0.15
SEMNi/AVS	-0.19	1	0.27	0.85
SEMNi-AVS	0.71	0.27	1	0.65
(SEMNi-AVS)/*f*OC	0.15	0.85	0.65	1

Table 2-8. Final multiple linear regression models of numerically dominant invertebrate responses and total invertebrate taxa to total sediment (SEM_{Ni}-AVS/$f$oc). Initial full model (Biotic response = SEM_{Ni}-AVS/$f$oc + Sediment Type + Date + (SEM_{Ni}-AVS/$f$oc): Sediment Type + (SEM_{Ni}-AVS/$f$oc):Date) reduced through iterative model reduction until a final model with all significant independent variables was obtained.

					Overall Model:			
Response	**Coefficients**	**Value**	**T**	**P(>t)**	**r^2**	**F**	**df**	**P**
Total Density	Intercept	9.3	111.1	<0.001	0.53	25.7	2, 45	<0.001
	SEMNi-AVS/$f$oc	-0.005	-3.39	0.001				
	Sediment Type	-0.76	-6.5	<0.001				
Chironomidae	Intercept	11.8	10.4	<0.001	0.54	17.3	3, 45	<0.001
	SEMNi-AVS/$f$oc	-0.005	-3.13	0.003				
	Sediment Type	-0.79	-6.04	<0.001				
	Date	-0.33	-2.46	0.018				
Hyalellidae	Intercept	6.78	23.4	<0.001	0.38	13.9	2,45	<0.001
	SEMNi-AVS/$f$oc	-0.017	-3.22	0.002				
	Sediment Type	-1.79	-4.36	<0.001				
Total Taxa	Intercept	4.53	7.29	<0.001	0.35	7.74	3, 44	<0.001
	SEMNi-AVS/$f$oc	-0.002	-2.53	0.023				
	Sediment Type	-0.17	-2.3	0.026				
	Date	0.27	3.78	<0.001				

Table 2-9. EC_{10} and EC_{50} estimates and 95% confidence intervals for dominant invertebrate taxa, densities, and total taxa. All threshold values are reported in mg/kg of Ni.

			EC10			EC50		
Predictor	**Sediment**	**Response**	**Estimate**	**95% CI**	**95% CI**	**Estimate**	**95% CI**	**95% CI**
Total Ni (mg/kg)	MR	Total Density	137	112	169	167	149	187
		Chironomidae	139	106	183	172	144	207
		Crangonyctidae	10	0	1.7x107	113	2.6	4840
		Hyalellidae	No solution can be calculated					
		Elmidae	0.2	0	5.9x108	13	0	5.8x106
		Total Taxa	112	68	185	185	134	256
	WD	Total Invertebrates	301	26	3489	4509	1644	12370
		Chironomidae	290	7	12129	5585	1172	26622
		Crangonyctidae	946	290	3088	2041	431	9656
		Hyalellidae	778	54	11138	3392	1054	10913
		Elmidae	75	0.7	7645	1174	213	6474
		Total Taxa	2055	114	36938	19965	1186	335959
	Combined	Total Density	124	21	727	1441	629	3304
		Chironomidae	131	19	920	1523	599	3871
		Crangonyctidae	106	3	4256	601	111	3243
		Hyalellidae	221	52	944	910	303	2734
		Elmidae	4.0x107	0	1.9x1014	0.2	0	3.0x1013
		Total Taxa	370	0.2	710158	27351	636	1.2x106
(SEMNi-AVS)/$f$oc	MR	Total Density	1432	824	2487	2566	1737	3790
		Chironomidae	1679	1006	2803	2400	1870	3082
	WD	Total Density	96	0.5	17353	1701	310	9331
		Chironomidae	174	0.7	43297	1844	298	11417
	Combined	Total Density	No solution can be calculated					
		Chironomidae	No solution can be calculated					
SEMNi/AVS	MR	Total Density	21	7	65	88	41	189
		Chironomidae	15	0.6	341	63	33	120
	WD	Total Density	0.02	0	89	5	0.3	85
		Chironomidae	0.04	0	724	7.6	0.3	202
	Combined	Total Density	No solution can be calculated					
		Chironomidae	No solution can be calculated					

Figure 2-1. Streamside mesocosm at Warden Ditch summer of 2007.

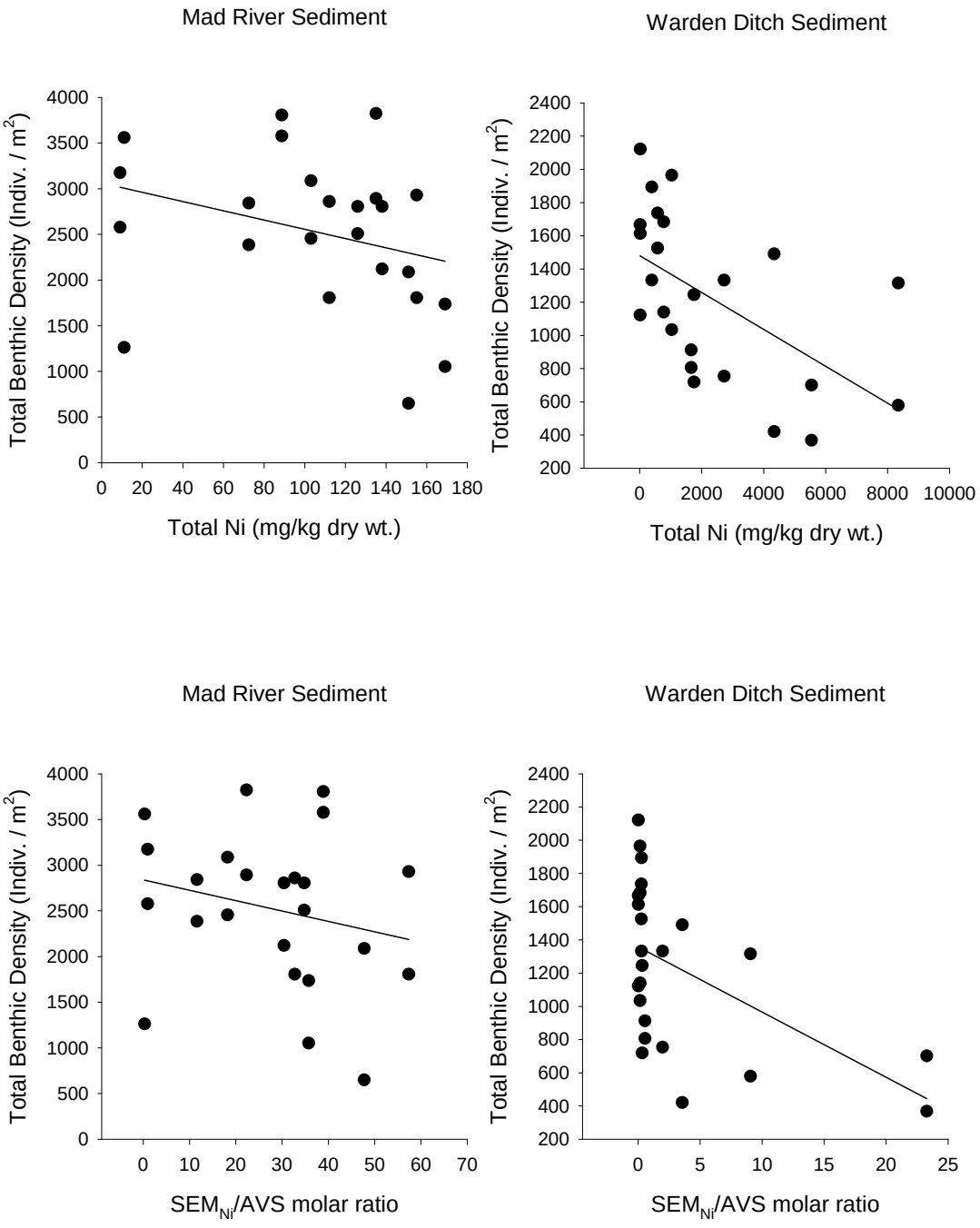

Figure 2-2. Total benthic density responses vs. total Ni on 30-Aug-07, separated by sediment type (MR and WD).

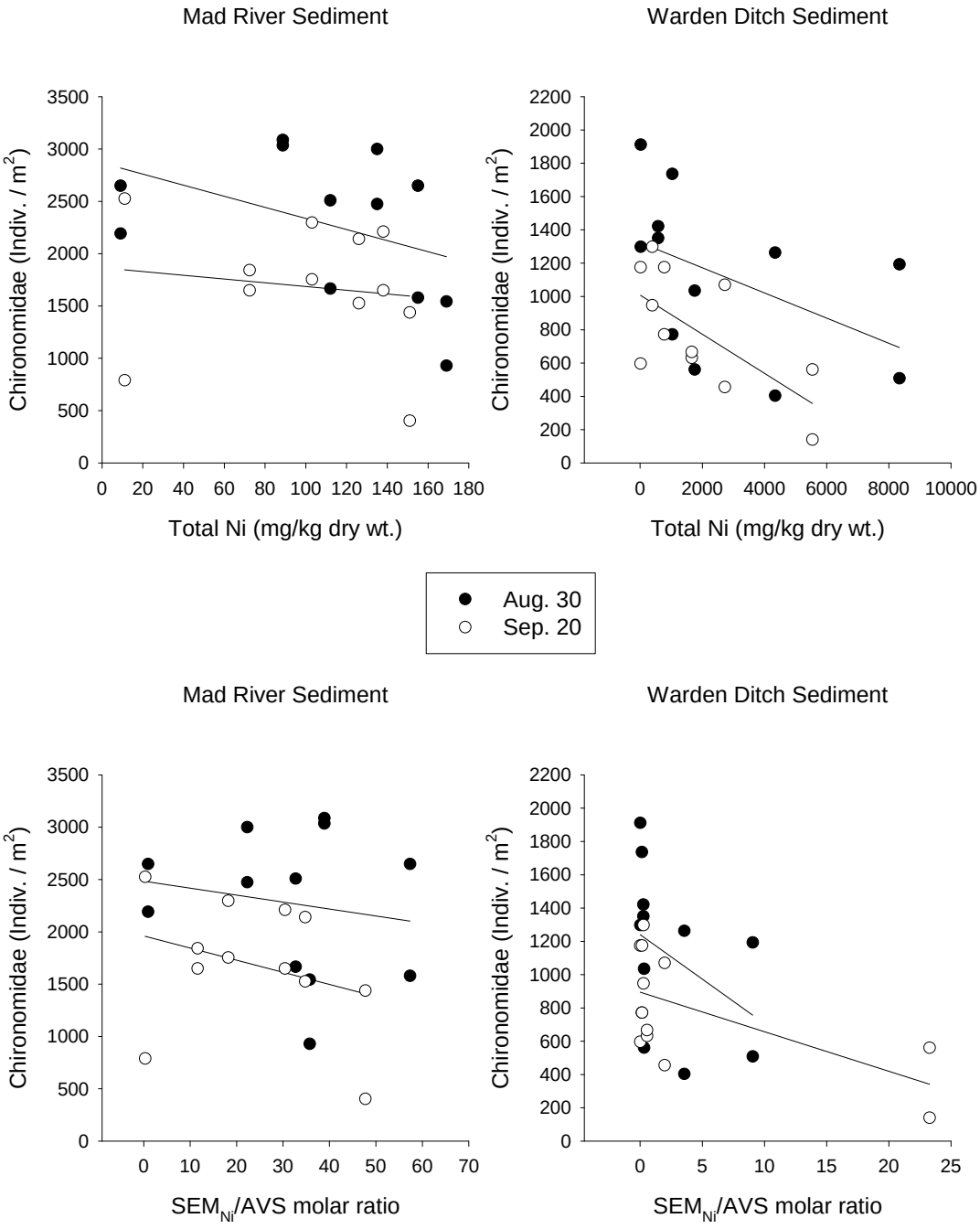

Figure 2-3. Chironomidae responses to total Ni and SEM/AVS model on both sampling dates 30-Aug-07, and 20-Sept-07, separated by sediment type (MR and WD).

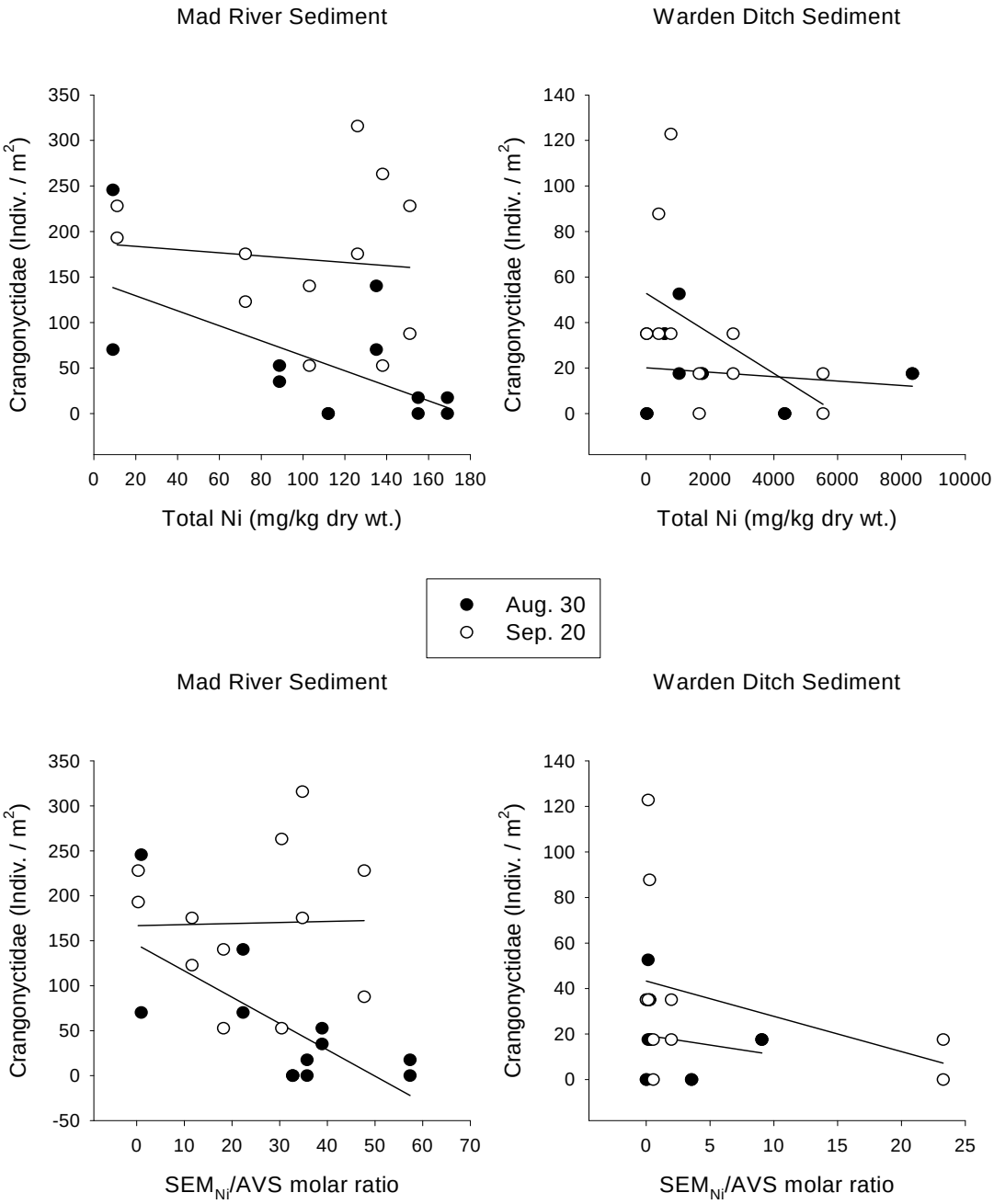

Figure 2-4. Crangonyctidae responses to total Ni and SEM/AVS model on both sampling dates 30-Aug-07, and 20-Sept-07, separated by sediment type (MR and WD).

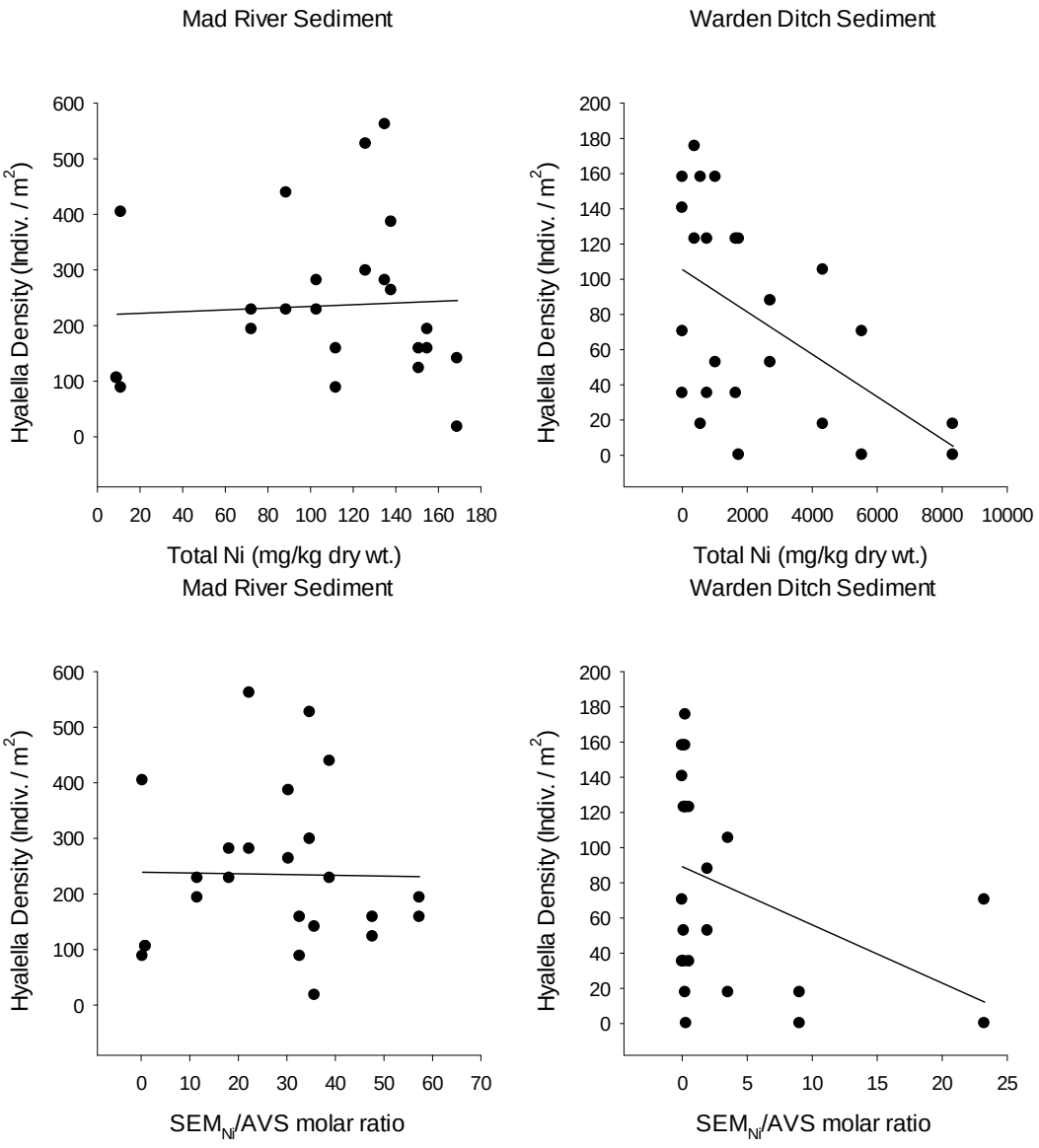

Figure 2-5. Hyalella responses to total Ni and SEM/AVS model on 30-Aug-07, separated by sediment type (MR and WD).

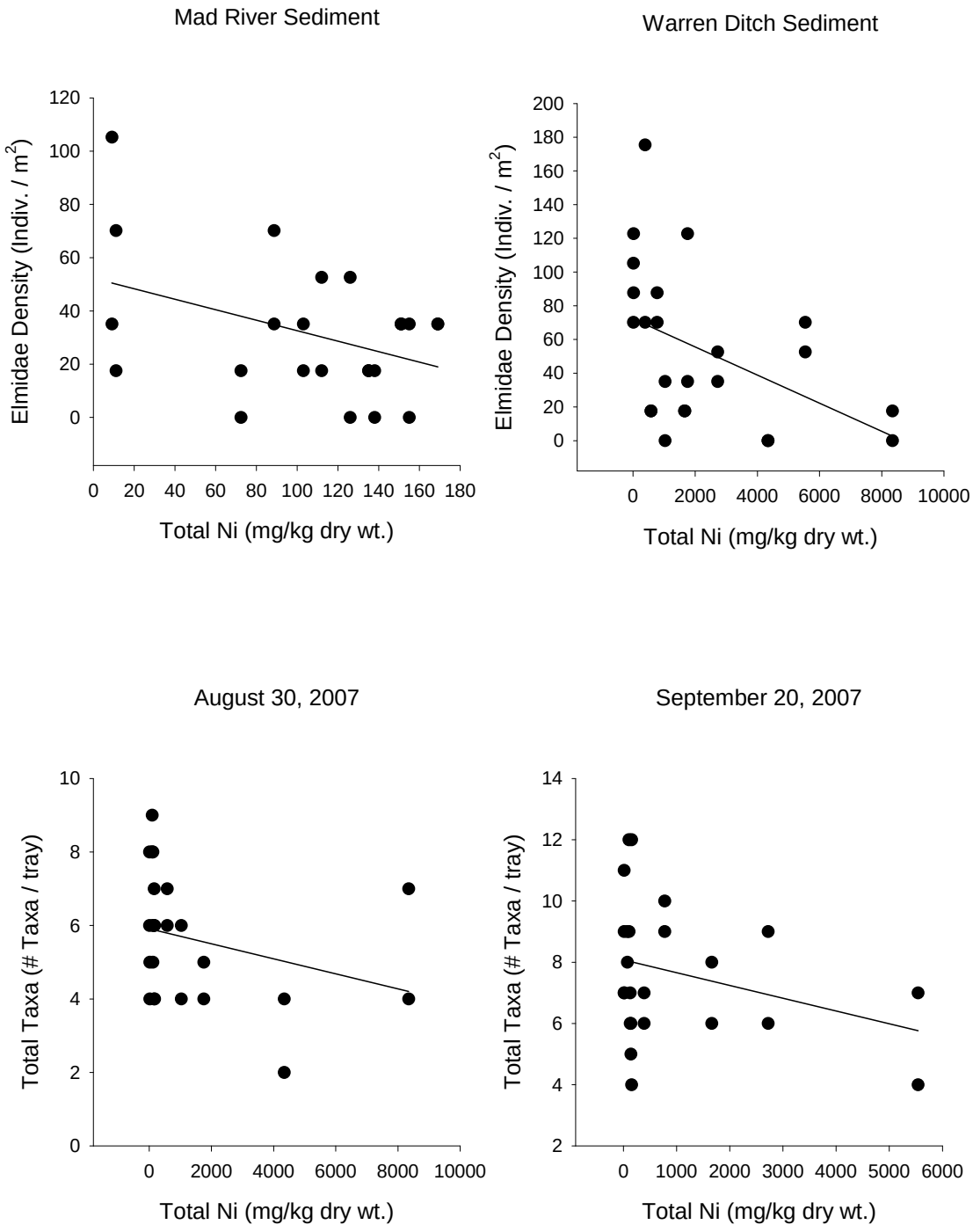

Figure 2-6. Elmidae and Total Taxa responses to total Ni on both sampling dates 30-Aug-07 and 20-Sept-07, separated by sediment type (MR and WD).

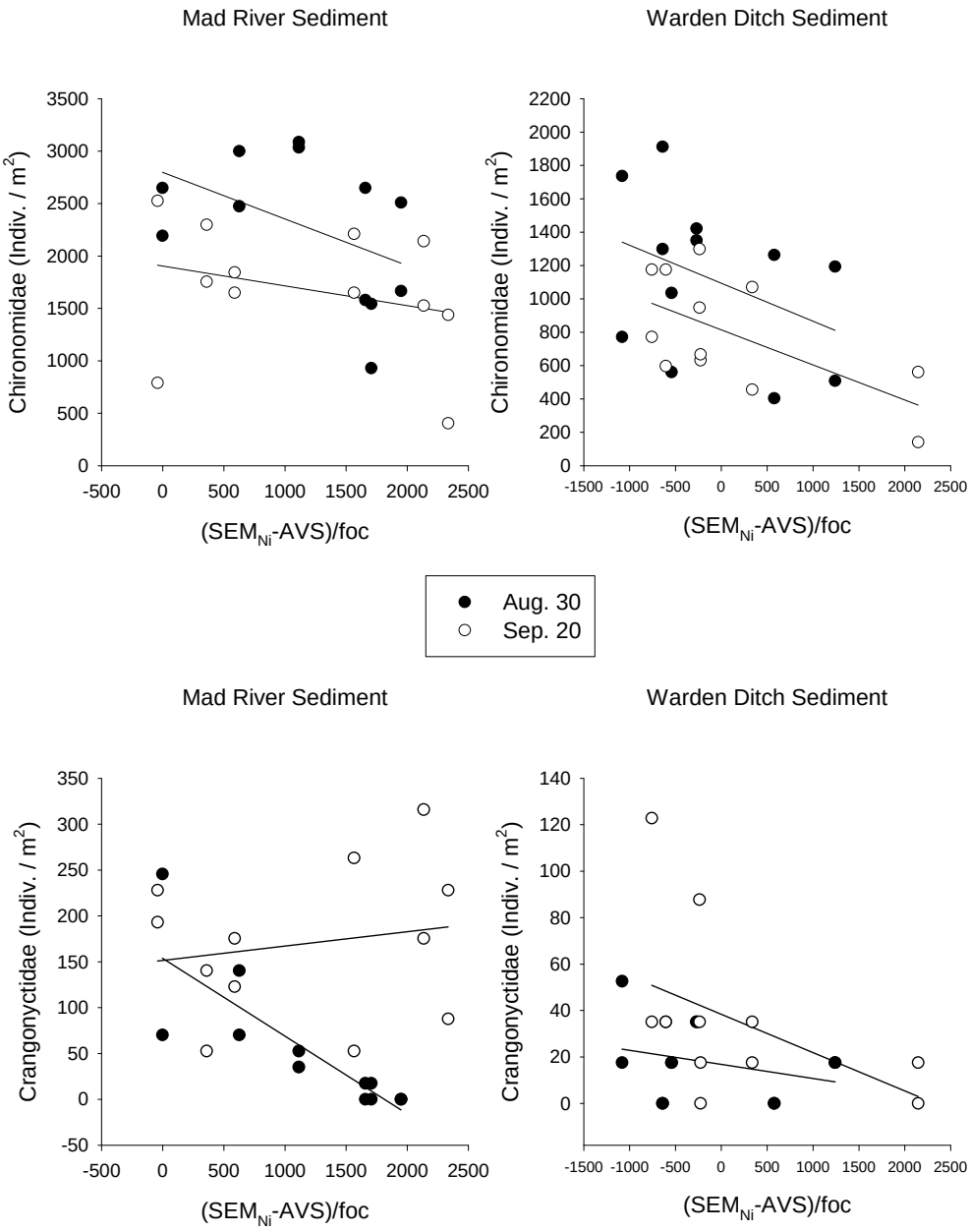

Figure 2-7. Chironomidae and Crangonyctidae densities vs. (SEM_{Ni}-AVS)/*foc* on both sampling dates 30-Aug-07 and 20-Sept-07, by sediment type.

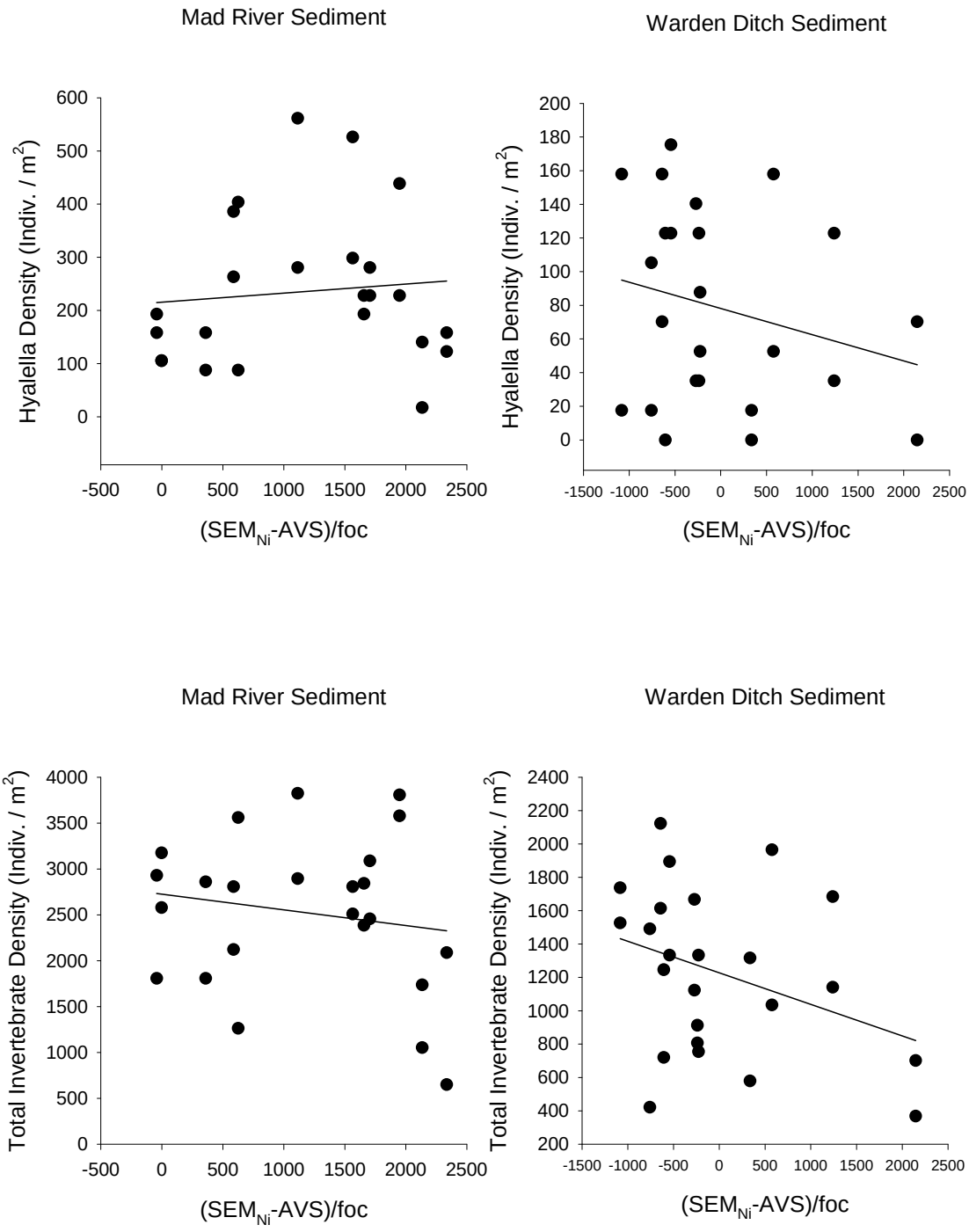

Figure 2-8. Hyalella and Total Invertebrate densities vs. (SEM_{Ni}-AVS)/*foc* on 30-Aug-07, by sediment type.

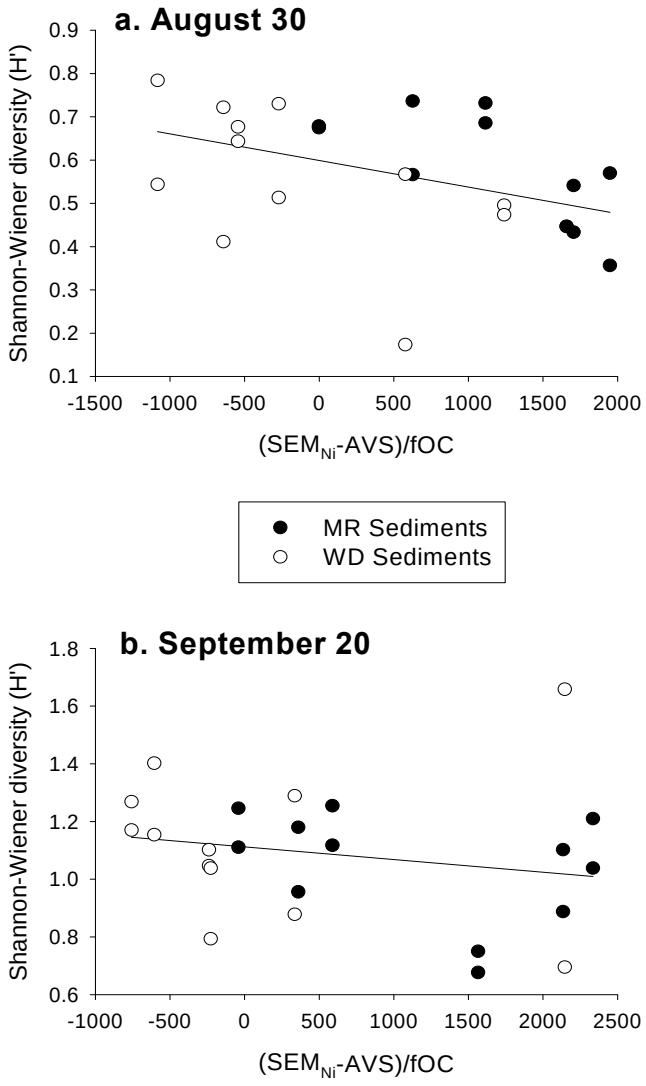

Figure 2-9. . Shannon-Wiener diversity (H') in response to OC normalized excess SEM_{Ni}, by dates (30-Aug-07 and 20-Sept-07).

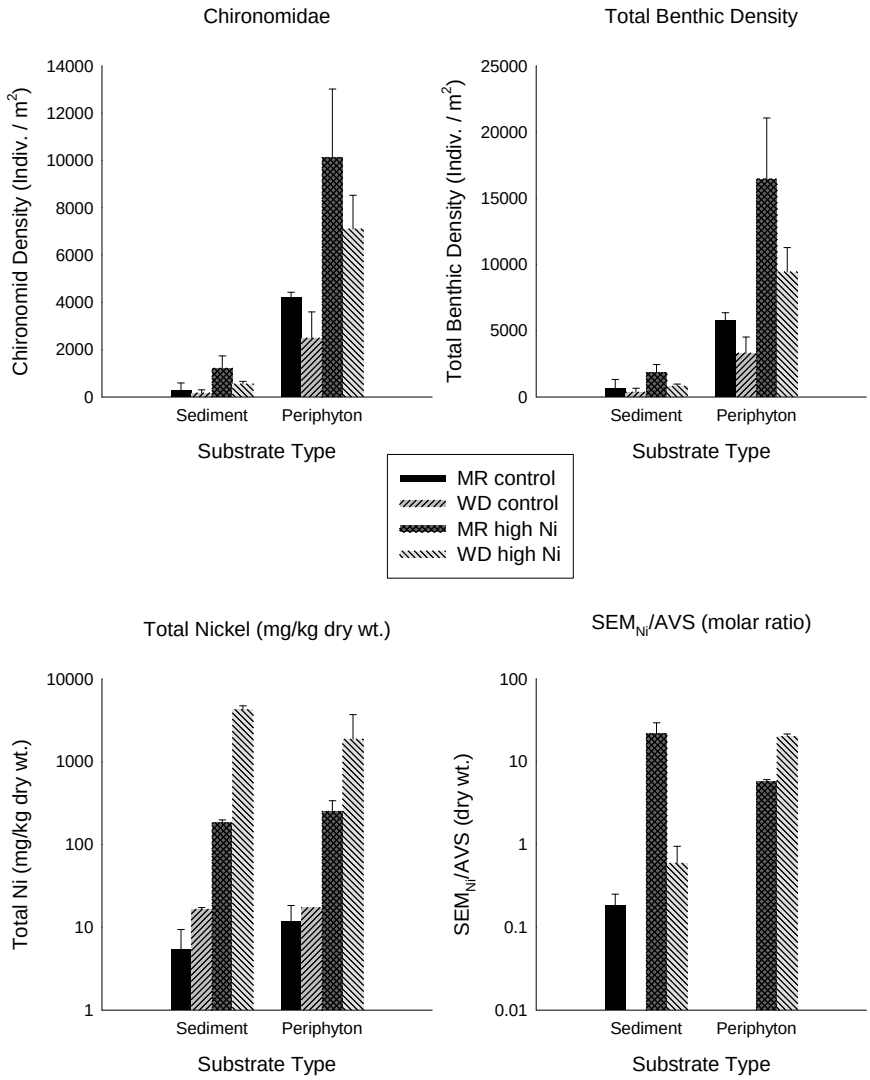

Figure 2-10. Chironomid densities, total benthic densities, total Ni concentrations, and SEM_{Ni}/AVS by sediment type, substrate type, and nickel treatment level (control vs. highest spiking concentration), for 27-Nov-07 benthic colonization trays. Vertical bars represent mean values +1 standard deviation. Total Ni and SEM_{Ni}/AVS levels are represented logarithmically for visualization purposes.

CHAPTER 3 – MACROINVERTEBRATE EFFECTS OF SEDIMENT NICKEL EXPOSURES IN A STREAMSIDE MESOCOSM ON THE STILLWATER RIVER (2008)

1-0 ABSTRACT

Nickel bioavailability in sediments may be detrimental to aquatic insect growth and benthic macroinvertebrate community structure when Ni levels exceed ecological effect threshold limits. Transplanted benthic macroinvertebrate communities were exposed in a streamside mesocosm for 4 wks near Stillwater River, Ohio, USA. The objectives of this study were to evaluate factors that controlled Ni bioavailability and how transplanted benthic macroinvertebrate communities responded to a series of Ni-spiked sediments. Benthic macroinvertebrate communities were exposed to a dilution gradient of Ni (5 concentrations, plus a reference) on two sediment types (high AVS, OC, and low AVS, OC). Benthic communities and sediment chemistry were collected at 14 d and 28 d. The benthic communities responded negatively to increasing Ni concentrations. Multiple regression analyses showed correlations with SEM/AVS models, sediment chemistry variables (Total Mn, SEM_{Ni}, total organic carbon, AVS) and a host of benthic metrics (total taxa, number of EPT Taxa, % Ephemeroptera, % EPT, Caenidae, Heptageniidae, and others). Ni flux from sediments was observed from both sediment types as early as 14 d, and continued throughout the study to 28 d. This pattern of Ni flux has been seen in other field and laboratory studies. *Hyalella azteca* and *Chironomus dilutus* were exposed for 4 d within < 48 h of Ni sediment deployment, and

showed no acute toxicity effects. Ni field sediment exposures and toxicity was related to the metal complexation capacity of the sediments, and SEM/AVS differences were observed between the sediments with high AVS/TOC compared to sediments with the low AVS/TOC.

2-0 INTRODUCTION

Bioavailability of metals is of major concern for water and sediment toxicity tests (Di Toro 2001, 2005), and a thorough understanding of all the possible partitioning phases for the metal is critical to understanding toxicity (Callendar 2003). The most toxic form of heavy metals is the free divalent state (Ni^{2+}, Cu^{2+}, Zn^{2+}, Pb^{2+}, and Cd^{2+}) (Callendar 2003). Physico-chemical processes (i.e. redox and pH) can change the speciation of metals in streams and rivers (Gaillardet 2003), and these processes can be biologically or chemically mediated within a system (Callendar 2003, Goldhaber 2003). Changes in pH and redox can cause metals to be released from one solid phase, and possibly scavenged or sequestered by another solid phase (Miao et al. 2006, De Jonge 2012). Sediment metal chemistry is complex, and when added with the dynamics of aquatic systems and benthic organisms, results can provide a setting for complexed conclusions.

Few studies (Chapter 2, Nguyen et al. 2011, Costello et al. 2011) have been conducted which provide realistic sediment toxicity threshold effect levels for nickel exposures to freshwater organisms. Reproducing natural conditions that are important in

the determination of accurate nickel threshold levels is difficult in the laboratory. As seen in numerous studies (Di Toro et al. 1996; Boothman et al. 2001; Rickard and Morse 2005; USEPA 2005; Prasad et al. 2006; Gomez-Alvarez et al. 2007), acid volatile sulfide (AVS), iron and manganese oxyhydroxides, and organic carbon can fluctuate in sediments, but are easily affected by laboratory manipulations which alter redox conditions, pH, sediment microgradients, and partitioning/flux to overlying waters. Potentially these changes can alter the bioavailability of nickel and subsequent partitioning to other phases (i.e. solid, water, or air) (Callendar 2003).

Community tests using natural sediments spiked with metals (Ni and four others) have been studied by numerous researchers (Boothman et al. 2001, Burton et al. 2005b, Costello et al. 2011, Nguyen et al. 2011). Results have varied, Boothman et al. (2001) found no differences in benthic communities between reference sediment and metal spiked treatments, Nguyen et al. (2011) and Costello et al. (2011) showed decreasing benthic diversity effects with increasing Ni. Lee and Lee (2005) found that Ni spiked sediments showed an increase in Ni tissue concentrations in *Neanthes arenaceodentata*, but was not related to the SEM/AVS model. These studies are indicating variable results when exposed to sediments amended with Ni. The need to show benthic response to increasing Ni is warranted, and appears to be dependent upon bioavailable Ni.

The bioavailability of nickel associated with sediment phases is significantly affected by AVS, OC, Fe and Mn oxides , and therefore, accurate threshold effect levels

must be conducted under more realistic exposure conditions in a field setting. A streamside mesocosm was developed in Chapter 2, and this system delivered natural stream water continuously for 4 wks. Natural sediments high and low in AVS and OC were spiked with nickel were placed in the streamside mesocosm. This system was developed to utilize natural stream water, and other environmental variables to provide more realistic exposures to benthic macroinvertebrate communities to Ni amended sediments.

2-1 Objective - The objectives of this study were to examine how field collected benthic macroinvertebrate communities responded to a gradient of Nickel (1200-7000 mg/kg) spiked in two different sediment types in a streamside mesocosm, and determine how Ni bioavailability is affected.

2-2 Hypothesis: Macroinvertebrate communities will respond negatively to increasing Ni, and sediment with low AVS and OC content will have most bioavailable Ni over time.

3-0 MATERIALS & METHODS

3-1 *Streamside Mesocosm Site and Design*

The streamside mesocosm was deployed on the bank of the Stillwater River (Covington, Ohio, USA), and this section of the Stillwater River attains the Ohio EPA highest water quality designation (Exceptional Warmwater Habitat). The mesocosm dimensions and system set up are described in Chapter 2 (section 3-2). Water from the

Stillwater River was delivered via an electrical pump that ran continuously for 4 wks. The channel design, water volume, and flow-thru characteristics are described in Chapter 2 (section 3-2, Fig 2-1).

3-2 Sediment Collection and Spiking and Deployment

Sediments were collected from Big Beavercreek (BC) and Greenville Creek (GC), and stored at 4°C until needed for spiking. This study used similar sediment types that were used in the Chapter 2, BC sediments (high AVS, TOC) and GC sediments (low AVS, TOC), and these sediments were chosen based on objectives of the study. Sediments used in Chapter 2 were WD (high AVS, TOC) and MR (low AVS, TOC). Sediments were spiked with $NiCl_2 \bullet 6H_2O$ (Fisher Scientific, Pennsylvania, USA) in a serial dilution series (5 concentrations plus a reference) based on dry sediment weights. Sediment dry weights and equilibration times were followed as described in Chapter 2 (section 3-3).

At the site, sediments were deployed on 23-Jul-08 and loaded into mesh lined trays (25 cm L x 7.6 cm W x 6.3 cm H). Four replicate trays were assigned to each sediment treatment, resulting in a total of 48 trays for the study (6 treatments x 2 sediment types x 4 replicates/treatment). Each channel received 24 trays of a single sediment type, arranged so that sediment Ni concentrations increased from upstream to downstream. Two channels were used, and one received GC sediments, and the other

channels received BC sediments. The positioning of the trays was the same for both sediment types.

3-3 Benthic Invertebrate Collection, Deployment, and Identification

On 24-Jul-08, benthic invertebrates were collected from two nearby streams within Southwest Ohio, USA (Great Miami River and Greenville Creek) using the same methods as described in Chapter 2 (section 3-5). Macroinvertebrates were transported, sorted, and introduced to the sediment trays following the same methods described in Chapter 2 (section 3-5). A total of three day zero benthic replicates were chosen on 24-Jul-08 for QA/QC purposes. These samples were preserved in 90% ethanol, and sent to Great Lakes Environmental Corporation (GLEC) (Traverse City, Mi) for benthos identification to family level.

All reference and highest Ni treatments were sorted and identified in house. All other Ni treatments were sorted and identified by GLEC to the lowest practical level for non-insects (order or family), with chironomids and other insects being identified to family level using Merritt et al. (2008). Invertebrate samples were sorted using either a 10x dissecting microscopes or 4x magnifying lens, and subsampling was not required for any samples. The samples for GLEC were sieved and sorted and captured Nematoda, and samples in house were not sorted to this taxonomic group. This taxonomic group was not used in any of the statistical analyses.

3-4 Sediment Sampling, Sediment and Water Chemical Characterization

Sampling of the sediments was performed 7-Aug-08, (2 wk), and 21-Aug-08 (4 wk). During the 2 week and 4 week sampling, two trays were removed from each treatment, and benthos and sediment chemistry samples were taken, following the methods described in Chapter 2 (section 3-6). All water and DOC samples were collected in acid-cleaned centrifuge tubes, and were preserved and filtered as described in Chapter 2, sec 3-7.

All sediment total metal digestions were performed in Teflon digestion vessels (Savilex, Eden Prairie, MN, USA). Dried sediments were added to each vessel along with concentrated HNO_3 and HCl acid (3:2 volume) (USEPA 2007). Solutions were then analyzed on a Flame Atomic Absorption Spectrophotometer for totals Ni, Fe, and Mn. Procedural blanks, analytical blanks, and standards were used for QA/QC.

The AVS and SEM_{Ni} analyses were determined following methodology in USEPA (1991). The AVS method extracts SEM_{Ni} with 1M HCl, which breaks the metal-sulfide bond and releases H_2S (g) and Ni^{2+} (SEM_{Ni}). The sulfide is then trapped as S^{2-} in a NaOH solution. This sulfide concentration was colorimetrically analyzed on a Thermo Scientific Spectrophotometer (Fisher Scientific, PA, USA), and the remaining SEM_{Ni} was filtered and analyzed on a Perkin Elmer Flame AA or PerkinElmer ICPMS (PerkinElmer MA, USA). Procedural blanks, analytical blanks, duplicates, and spikes were used for QA/QC in AVS analyses, and procedural blanks, analytical blanks, and standards were

used for QA/QC in the SEM_{Ni} analyses. All sediment chemical concentrations are presented as concentration on a dry weight basis.

Sediment % solids and total organic carbon (TOC) content was performed by following methods outlined in Heiri et al. (2001) and Santisteban et al. (2004). Sediment samples (5-10 g) were dried for 24 h at 105°C. Dried sediments were homogenized with a mortar and pestle to a fine grained sample. Approximately 1 g of dried sediment was added to ashed (550°C) crucibles, and then burned at 550°C for 4 h. Sample was weighed after 4 h, and loss on ignition (LOI) of total organic carbon was determined by methods outlined in Heiri et al. (2001), and a correction factor of 0.38 was used to convert LOI to organic carbon (Redfield 1934).

3-5 Physico-chemical and sediment pH monitoring

Mesocosm physico-chemical parameters dissolved oxygen (DO), conductivity, temperature, pH, hardness, alkalinity, DOC, total water Ni, and sediment pH were taken at each deployment and retrieval time point. During deployment, sediment collection, and site visits sediment pH and sediment temperature were recorded with an YSI pH 100 meter. Measurements were taken in the surficial sediment (SS) layers (1-2 cm), and in the deep sediment (DS) layer (5-6 cm).

3-6 In situ toxicity testing

The *in situ* toxicity testing chambers used in this study were adapted from after Burton et al. (2005a). The chambers had 250 μm nylon mesh windows to facilitate water and sediment exchange while deployed on the sediment trays. Four replicate chambers were placed against the sediment on the reference and the three highest Ni concentrations for each sediment type (GC and BC), and four additional replicate chambers were placed in the water column on each respective treatment. Ten *H. azteca* and *C. dilutus* were placed in the same chambers, and exposed for 96 h starting on 1-Aug-08.

3-7 Data analysis

A host of benthic metrics (e.g. number of EPT, % EPT, % Ephemeroptera) and diversity indices (e.g. Shannon Diversity Index, Pielou's J, Simpson's Index) were calculated with a total of 39 such indices and metrics being used. All statistical analyses were run on SAS 9.2 or Minitab 16.

A multiple linear regression was used to determine relationships between the dependent variables (metrics and indices) and independent variables (sediment and water chemical variables). Sediment and water chemical variables were ln + 1 transformed, count data was square root + 0.5 transformed, and proportional data was arcsine transformed following recommendations in Zar (1999). The terms date and sediment type (GC or BC) were used as categorical variables.

A step-wise regression with backward elimination was used to determine term(s) selection in the model. When regression models were found to be significant ($\alpha < 0.05$),

multicollinearity was determined by using a variance inflation factors (VIF) criteria of < 4 to determine if terms were collinear (Pan and Jackson 2008; O'Brien 2007). A test for Heteroscedasticity (Chi-square $\alpha < 0.05$) was also used for model selection, and then an interaction term was added and analyzed again with the following criteria (Zar 1999).

One-way ANOVA with Dunnett's test was used on selected benthic metrics to determine no effect levels for the SEM/AVS models. The BC or GC reference values were used to calculate the control values.

Sediment chemistry assumptions described in Chapter 2 (section 3-9) were also followed in this study. Experimental replicates were collected for each sediment type and treatment; however, analytical replicates on these treatments were not performed on the individual sediment trays. The sediment chemistry variables (total Ni, total Fe, total Mn, SEM_{Ni}, AVS, and TOC), and the SEM_{Ni}/AVS models used in the regression models were all assumed to be similar from the replicated tray. Since only two trays were collected at each time point, one tray was frozen for SEM_{Ni}/AVS, and the other stored at 4°C for total metals and TOC. It is important to understand these assumptions eliminate variance between replicates, and this approach could possibly overestimate any regression models and subsequent models of bioavailability. However, due to experimental and logistical constraints these assumptions were necessary to follow.

For all *in situ* toxicity testing results, the same statistical methods and assumption tests were used as described in Chapter 2 (section 3-9). Any replicated data presented in tables and graphs are means ± standard deviations.

4-0 RESULTS AND DISCUSSION

4-1 Sediment chemistry and bioavailability

Sediment chemistry was characterized in both sediments and through time. The BC sediments had high concentrations of AVS (24-27 μmol/g), TOC (3.4-5.5 %), and total Fe (242-326 μmol/g) in the reference and Ni treatments (Day 0, Table 3-1). In contrast, GC sediments contrasted with low AVS (0.06-0.08 μmol/g), TOC (0.4-2.4 %), and total Fe (95-124 μmol/g) in the reference and Ni treatments (Day 0, Table 3-2). Total Mn was similar in both BC (5-6 μmol/g) and GC (7-8 μmol/g) treatments (Day 0, Tables 3-1 and 3-2). Although particle size distribution was not performed, BC sediments were depositional sediments, black in color (clay and silt), and the GC sediments were erosional sediments tan in color (gravel and sand).

The distinct differences in these sediment types have been previously observed in metal toxicity (Chapter 2, Burton et al. 2005, Costello et al. 2011, Nguyen et al. 2011) tests, and differences in sediment chemistry has helped to discern differences in metal bioavailability. The BC and GC sediment selection for this study provided a low and high range of principal sediment chemical parameters (AVS, TOC, Fe) which have been identified as important factors in controlling metal bioavailability (Di Toro et al. 2005, USEPA 2005).

There were noticeable differences in bioavailability of Ni between BC and GC sediments. Day 0, BC sediments had three Ni treatments with negative (SEM_{Ni}-

AVS)/$f$oc values, and at 28 d this increased to four concentrations (Table 3-1). The SEM_{Ni}/AVS values followed the same trend at Day 0 and 28 d, with these concentrations being < 1 (Table 3-1). In comparison, the GC sediments had no Ni or reference treatments with (SEM_{Ni}-AVS)/foc values below 0 (Table 3-2), and the SEM_{Ni}/AVS values for all the GC treatments were > 1 (Table 3-2). The highest (SEM_{Ni}-AVS)/foc for BC sediments was 1216 μmol/g (Day 0), and GC sediments was 1875 μmol/g (28 d) (Tables 3-1 and 3-2).

Characterizing the bioavailability of Ni in both sediments was needed to discern benthic responses to these increasing Ni concentrations. The majority of the Ni treatments in the BC sediments all showed relatively low concentrations of bioavailable Ni, and GC sediments had much higher concentrations of bioavailable Ni with the (SEM_{Ni}-AVS)/foc and SEM_{Ni}/AVS models being below the theoretical threshold limits, < 130 μmol/g and < 1, respectively (Di Toro et al. 2005, USEPA 2005). These two sediment types contrasted in AVS and TOC content similar to the ones used in Chapter 2, but the AVS content was much lower in BC than WD.

4-2 Dissolved organic carbon and total organic carbon

A trend in both BC and GC Day 0 samples showed that TOC (%) was positively related Ni concentration, but this trend was not seen in the 14 or 28 d samples. In BC reference treatment, the TOC was 3.5 %, and in the highest Ni treatment (BC 7060) TOC was 5.4%. In the GC reference treatment TOC was 0.5% and the highest Ni treatment

(GC 1254) was 2.4%. In both BC and GC sediments, the increase from reference to the highest Ni treatment was 1.9% (Tables 3-1 and 3-2).

The TOC increase may have been an artifact of the loss on ignition (LOI) methodology (Heiri et al. 2001) with high Ni amendments to sediments. These same TOC increases were not seen in the TOC samples in Chapter 2 (Tables 1-1a,b), and these were analyzed on a carbon analyzer, and not burned in a muffle furnace.

4-3 Physico-chemical variables and sediment porewater pH

Physico-chemical variables were measured throughout the study were similar throughout the study. Temperature (23.2 ± 1.5 °C), DO (6.8 ± 0.4 mg/L), conductivity (701 ± 59µS/cm), and pH (7.91 ± 0.14) varied little during the study (Table 3). Dissolved organic carbon was 3.4 ± 1.2 mg/L, and hardness was 290 ± 26 mg/L of $CaCO_3$ (Table 3-3).

These physico-chemical variables showed no signs of stress on the system, and these were similar to the readings during the 2007 streamside exposure (Chapter 2, Table 2-2). This is demonstrating that during the exposure periods the streamside mesocosm was functioning, and not adding stress to the system by affecting the physico-chemical parameters.

Sediment pH was measured in reference and the highest Ni treatments in both BC and GC sediments. Sediment pH and temperature was taken at surficial sediments (SS) < 2 cm deep, and in deep sediments (DS) > 2 cm in the sediment trays. Sediment pH

declined with depth and with increasing Ni concentration (Table 3-4). Sediment pH was higher in GC sediments, and GC reference had more circumneutral values in both SS and DS, 7.48 ± 0.29 and 7.36 ± 0.23, respectively (Table 3-4). The BC 7060 Ni treatment had the lowest pH readings, and the pH was slightly acidic in the DS (6.32 ± 0.27) (Table 3-4). The DS in the BC 7060 represented the lowest pH of all the measured treatments (Table 3-4). The DS in both BC and GC sediments had ~0.5 lower pH than SS.

These increased pH values in the SS may be a function of the surficial layer becoming more oxic from bioturbation (Goldhaber 2003). In this sediment-water interface, it has been shown that metals can be released from their metal-sulfide bonds, and become more bioavailable (Miao et al. 2006). During these conditions metals can flux up from bottom sediments into the sediment-water interface (Amatya and Mika 2008). There were no attempts at determining Ni depth profiles, however, the pH measurements were lower in the DS than SS and suggests higher Ni concentrations, and more sulfate reducing bacteria (SRB) activity (Goldhaber 2003).

4-4 Ni-sediment flux and sediment chemistry changes

Ni diffusion from the sediment to the water was documented with DGT and sediment data in Chapter 2. In this study Ni flux was observed in both sediment types, but GC sediments lost more total Ni over time than did BC sediments (Table 3-5). The percent change from Day 0 and either 14 d or 28 d was significant over time for total Ni and SEM_{Ni}. The BC Ni treatments showed less total Ni loss than the GC Ni treatment,

and these loses were more consistent at 28 d. At 14 d BC treatments showed a range 0 - 25% loss, with the BC 768 treatment showing a 1% gain. At 28 d collection, the range was 0 - 31% loss, with no treatments showing a gain (Table 3-5). At 14 d the GC treatments experienced total Ni loss from 0-50%, and the GC 110 treatment having a 29% gain (Table 3-5). At 28 d, the GC treatments total Ni loss ranged from 0 – 48%, with GC 110 and GC 460 treatments experiencing gains of 15% and 7 %, respectively. At 14 d, the SEM_{Ni} in the BC treatments was showing significant changes ranging from a BC reference gain of 65% to a 80% loss in BC 3262 (Table 3-5). These high SEM_{Ni} losses were also observed in GC sediments at both 14 d and 28 d, and with the exception of the reference treatments all Ni treatments in GC sediments experienced Ni flux at percentages ranging from 20-69% (14 d) and 27-71% (28 d) (Table 3-5).

The AVS, total Fe, and TOC all showed varied results when comparing Day 0 samples to 14 and 28 d samples (Table 3-5). The BC treatments at 28 d were different from all other BC and GC treatments, because these showed steady increases in AVS (46-55%) at 28 d. Total Mn had the most consistent values over time, and nearly all BC and GC treatments showed increases (Table 3-5).

Following Ni changes in the sediment type is important for characterizing Ni toxicity to benthic organisms. Ni flux out of both sediment types was evident at 14 d, and slowed when reaching 28 d. The GC sediments lost the most Ni over time, and the BC sediments had less Ni flux during the 28 d. These Ni flux and other sediment chemical parameter results were similar to those observed in Chapter 2 (Table 1-1c), and

other studies Boothman et al. (2001) and Naylor et al. (2006). Total Ni and SEM_{Ni} flux from sediments are most likely a function of spiking methods and short equilibration times (< 3 d). Liber et al. (2011) stated that there is no consensus on equilibration times, and they used a 10 d equilibration time. The current study Ni flux results contrast Costello et al. (2011) which used multiple staged spiking method, and longer equilibration times. This spiking method appears to be the preferred methodology for spiking Ni. Simpson et al. (2004) has demonstrated that care must be taken when spiking metals to sediments and equilibration times vary between metals (Cd, Cu, Zn, Ni, Pb), and Ni is > 30 d. This study followed the spiking methodology and equilibration times as described in Chapter 2.

The reduction in AVS appears to be a function of adding metals to sediments, and subsequent decrease in AVS content (Di Toro et al. 1996; Simpson et al. 2004). However, all BC treatments at 28 d increased in AVS, and Rickard and Morse (2005) have stated that AVS changes with depth, length, and time. These increases may have been a function of sediment equilibration in anoxic sediments which may have promoted SRB activity, and subsequent hydrogen sulfide increases (Goldhaber 2003, De Jonge et al. 2012).

4-5 In situ toxicity testing

To rule out possible stress to benthic organisms from Ni diffusion to overlying water, and *in situ* toxicity testing on both BC and GC sediments treatments with *Hyalella*

azteca and *Chironomus dilutus* was performed. These results showed no acute Ni toxicity during the 96 h exposures. *Hyalella azteca* had ≥ 92% in most reference and Ni treatments, and only one Ni treatment with 87% survival. *Chironomus dilutus* had ≥ 90% in most reference and Ni treatments, with only treatment at 84%.

The *in situ* results were similar to Chapter 2 results when *in situ* toxicity was not observed during the initial acute toxicity tests. Ni was fluxing from both sediment types (Table 3-5); however, the high hardness, moderate DOC, and other possible ligands may have complexed with the Ni and rendered it unavailable to the organisms. Other studies have suggested that high hardness, DOC, and other ligands have the ability to attenuate Ni toxicity (Pyle et al. 2002, Di Toro et al. 2001, Gaillardet 2003). However, in Chapter 2 a late *in situ* toxicity test with *C. dilutus* showed slight acute toxicity. These results were attributed to the colonization of blue-green algae on the sediment trays in the mesocosm. The current mesocosm study had no such algal colonies were present.

4-6 Macroinvertebrate community responses to Ni

Benthic macroinvertebrate communities were dominated by Chironomidae, Caenidae, Coenagrionidae, Elmidae, and Ceratopogonidae. Total abundance of all of the dominant taxa did appear to differentiate between BC and GC sediment types, with a clear preference of GC sediments. The total family level taxa list for this study was 42 with 19 taxa being insects, and EPT taxa were represented by 11 families. There were a

total of 3042 macroinvertebrates sampled, of which 2462 were from the family Chironomidae.

Benthic macroinvertebrate communities declined with increasing SEM_{Ni}/AVS, SEM_{Ni}-AVS, and (SEM_{Ni}-AVS)/*foc* numbers, in both sediment types and dates (Figs 3-1 – 3-5), and Tables 3-6 – 3-9. Numerous benthic metrics and specific taxa were in agreement with SEM_{Ni}/AVS model predictions seen in the Chapter 2 (section 4-6). This showed that benthic macroinvertebrates in both studies showed decreasing numbers with increasing SEM_{Ni}/AVS values (Figs 3-2 – 3-5).

Individual taxa demonstrated significant effects to increasing Ni and SEM_{Ni}/AVS models. Caenidae experienced decreasing numbers with increasing SEM_{Ni}/AVS values, with all the reference values being below the predicted no effect value of < 1 (Fig 3-2). The regression model showed a significant effect ($p < 0.0001$, $R^2 = 0.78$), and predicted that Caenidae responded negatively with increasing AVS and SEM_{Ni}/AVS models (Table 3-8). As total Ni increased in both sediment types, the number of Chironomidae declined, and had higher numbers at 14 d on GC sediment than BC sediments (Figs 3-1). Chironomidae showed a significant negative effect related ($p < 0.0001$, $R^2 = 0.71$) to increasing total Ni and Fe (Table 3-9). Heptageniidae demonstrated significant effects (($p < 0.0001$, $R^2 = 0.63$) showing a negative relationship between decreasing abundance and increasing SEM_{Ni}/AVS and total Fe (Table 3-8).

The number of Ephemeroptera taxa and number of EPT taxa demonstrated similar responses to increasing SEM_{Ni} (Table 3-7). These Ephemeroptera responses were

demonstrating a negative relationship with increasing SEM_{Ni} (Table 3-7). The number of EPT taxa showed no Ni effects at $SEM_{Ni}/AVS < 14.8$ μmol/g in GC sediments. The prediction of no Ni effects for the number of EPT taxa in BC sediments was $SEM_{Ni}/AVS < 0.013$ μmol/g at 14 d, and increased to 1.673 μmol/g by 28 d.

On noticeable trend was that numerous benthic metrics (% Sprawlers, % Burrowers) and diversity and richness indices (Shannon's Diversity, Hills Diversity Numbers N1, N2, and Margarlef Richness) showed significant regression relationships to SEM_{Ni}/AVS and TOC (Table 3-8). Only Simpson's Diversity and % Burrowers showed significant positive relationships with SEM_{Ni}/AVS and TOC (Table 3-8). These relationships were attributed to increase of Chironomidae in BC and higher Ni treatments on GC sediments.

The Ni sediment exposures in streamside mesocosm demonstrated that a host of benthic macroinvertebrate community indices and metrics responded negatively to increasing bioavailable Ni (SEM_{Ni}/AVS models). The three SEM_{Ni}/AVS model estimates were markedly different between the two sediment types, with model estimates being higher in GC sediments (Tables 3-6 – 3-8). This is suggesting that more Ni was bioavailable in GC sediments than BC sediments. In theory, Ni bioavailability should have been greater in the GC sediments, due to the low AVS and TOC content. The SEM_{Ni}/AVS models were also in agreement with other studies (Chapter 2, Nguyen et al. 2011) which states that chronic toxicity may be possible as SEM_{Ni}/AVS values increase to a threshold ($SEM_{Ni}/AVS > 8$, and $(SEM_{Ni}\text{-}AVS)/foc > 700$). The EqP method has

suggested using SEM-AVS in place of the SEM/AVS ratio, since its importance of adding the other partitioning phases (OC) (USEPA 2005). However, in the current study a host of benthic and diversity metrics were found to be significant in the multiple regression models with the SEM/AVS term. A majority of these responses were responding negatively with increasing SEM/AVS values. Total abundance, number of Ephemeroptera Taxa, Caenidae, Heptageniidae, and number of EPT taxa had some of the highest R^2 values (Tables 3-6 – 3-8). These responses also showed variation when looking at the $SEM_{Ni}/AVS < 1$ and $SEM_{Ni}\text{-}AVS \leq 0$ standard thresholds which predict lack of toxicity (USEPA 2005). The GC reference treatments with background Ni concentrations and nominal AVS content, thus had $SEM_{Ni}/AVS > 2$, and $SEM_{Ni}\text{-}AVS > 0$ in reference treatments. These values are higher than the other studies with no effect SEM_{Ni}/AVS thresholds, but the current study did have $(SEM_{Ni}\text{-}AVS)/foc < 150$ μmol/g reference values. In Chapter 2, substrate type (MR or WD sediment) was a common significant term in the regression analyses, but substrate type (GC and BC) was not a common significant term in this study.

There were increases in total taxa, total abundance and diversity on GC sediments vs. BC sediments. Some studies have suggested the importance of Fe and Mn oxides (Sundby 1994, Prasad et al. 2006, Costello et al. 2011, 2012), and the GC sediments is a sediment type that is more oxic and has moderate levels of Mn (gravely and sandy). In this study, Mn and Fe were not common significant terms as often as SEM_{Ni}/AVS

models, AVS, and TOC. This is suggesting that bioavailable Ni, TOC, and AVS is explaining most of the variation seen in the benthic community responses.

5-0 GENERAL CONCLUSIONS

The objectives of this study were achieved by showing how the benthic macroinvertebrate communities responded to a gradient of Nickel spiked in two different sediment types in a streamside mesocosm. This was likely due to two factors: Ni bioavailability (SEM/AVS models) differed markedly between the sediments resulting in different benthic exposures, and benthic communities showed a preference for the larger grained GC sediment.

Ni flux differences between sediment types (GC and BC), and the relationships between benthic responses and bioavailable Ni were similar to Chapter 2 results. Concentrations of Ni used in this study were relatively high when compared to natural environmental conditions found in most areas, but higher Ni sediment and water concentrations have been measured in Sudbury, ON region (Rasmussen et al. 2008). However, an increase in spiked Ni concentrations was needed to ensure Ni concentrations remained at high enough levels due to Ni loss during long-term exposures (i.e. Chapter 2). Ni spiking methods have improved since this study was performed, and Ni flux has lessened through newly developed spiking methodology and longer equilibration times (Costello et al. 2011).

The hypothesis was supported for this chapter, and benthic community structure was showing declines with increasing bioavailable Ni. In this study, EPT taxa were demonstrating Ni sensitivity, and GC sediments had higher bioavailable Ni. The GC sediments also demonstrated higher colonization than BC sediments. The benthic macroinvertebrate effects and supporting sediment chemistry (e.g. sediment pH, AVS, TOC, and SEM_{Ni}) provide useful data in support of SEM_{Ni}/AVS models, and demonstrate the need to understand how benthic communities change in the presence of Ni amended sediments. These benthos effects can have potential effects at higher trophic levels, and potential ecosystem effects.

Table 3-1. Big Beavercreek (BC) sediment chemistry variables and SEM/AVS models in the 2008 streamside mesocosm.

Ni Treatment Level	Collection Date	SEM_{Ni}/AVS (umol/g)	(SEMNi-AVS)/*foc* (umol/g)	(SEM_{Ni}-AVS) (umol/g)	Total Ni (mg/kg)	Total Ni (µmol/g)	SEM_{Ni} (µmol/g)	AVS (µmol/g)	Total Mn (µmol/g)	Total Fe (µmol/g)	TOC (%)
BC Reference	24-Jul-08	0.007	-740.69	-25.71	31	0.53	0.2	26	6	242	3.5
BC 516	24-Jul-08	0.283	-505.66	-17.02	516	8.79	7	24	6	243	3.4
BC 780	24-Jul-08	0.341	-498.57	-17.73	780	13.29	9	27	6	324	3.6
BC 1589	24-Jul-08	0.709	-173.72	-7.03	1589	27.07	17	24	6	325	4.0
BC 3262	24-Jul-08	1.693	367.55	16.61	3262	55.57	41	24	6	326	4.5
BC 7060	24-Jul-08	3.724	1216.75	66.26	7060	120.27	91	24	5	324	5.4
BC Reference	7-Aug-08	0.013	-646.86	-24.39	28	0.48	0.3	25	6	324	3.8
BC 516	7-Aug-08	0.108	-894.21	-26.24	386	6.58	3	29	6	343	2.9
BC 780	7-Aug-08	0.302	-661.90	-25.57	791	13.48	11	37	7	345	3.9
BC 1589	7-Aug-08	1.047	27.22	0.99	1588	27.05	22	21	6	324	3.6
BC 3262	7-Aug-08	0.404	-384.20	-12.07	2759	47.00	8	20	7	326	3.1
BC 7060	7-Aug-08	2.102	473.48	19.34	5425	92.42	37	18	6	326	4.1
BC Reference	21-Aug-08	0.008	-987.64	-37.46	31	0.53	0.3	38	7	346	3.8
BC 516	21-Aug-08	0.154	-785.57	-31.11	375	6.39	6	37	6	285	4.0
BC 780	21-Aug-08	0.356	-672.91	-26.38	550	9.37	15	41	7	345	3.9
BC 1589	21-Aug-08	0.609	-383.82	-14.45	1426	24.29	22	37	6	284	3.8
BC 3262	21-Aug-08	0.399	-537.29	-21.83	2267	38.62	14	36	8	367	4.1
BC 7060	21-Aug-08	1.673	580.97	23.91	5216	88.86	59	36	6	283	4.1

Simultaneously extracted metals (SEM)
Acid volatile sulfides (AVS)
Total organic carbon (TOC)

Table 3-2. Greenville Creek (GC) sediment chemistry variables and SEM/AVS models in the 2008 Streamside mesocosm.

Ni Treatment Level	Collection Date	SEM_{Ni}/AVS (umol/g)	(SEMNi-AVS)/*foc* (umol/g)	(SEM_{Ni}-AVS) (umol/g)	Total Ni (mg/kg)	Total Ni (µmol/g)	SEM_{Ni} (µmol/g)	AVS (µmol/g)	Total Mn (µmol/g)	Total Fe (µmol/g)	TOC (%)
GC Reference	24-Jul-08	1.640	9.68	0.05	22	0.4	0.1	0.08	7	117	0.5
GC 110	24-Jul-08	22.986	462.71	1.98	110	2	2	0.09	7	109	0.4
GC 255	24-Jul-08	55.669	718.85	3.88	255	4	4	0.07	6	119	0.5
GC 460	24-Jul-08	98.701	831.15	8.02	460	8	8	0.08	6	107	1.0
GC 763	24-Jul-08	166.649	887.30	13.43	763	13	14	0.08	7	124	1.5
GC 1254	24-Jul-08	510.187	1168.25	28.58	1254	21	29	0.06	6	95	2.4
GC Reference	7-Aug-08	3.595	30.76	0.15	22	0.4	0.2	0.06	6	108	0.5
GC 110	7-Aug-08	14.814	329.86	1.55	142	2	2	0.11	7	138	0.5
GC 255	7-Aug-08	45.055	457.13	2.61	240	4	3	0.06	7	142	0.6
GC 460	7-Aug-08	42.554	588.57	3.76	445	8	4	0.09	9	157	0.6
GC 763	7-Aug-08	112.071	1221.73	7.27	470	8	7	0.07	6	120	0.6
GC 1254	7-Aug-08	219.777	1402.45	8.87	631	11	9	0.04	7	145	0.6
GC Reference	21-Aug-08	2.068	13.65	0.09	21	0.4	0.2	0.09	8	151	0.7
GC 110	21-Aug-08	22.042	284.06	1.44	126	2	2	0.07	7	139	0.5
GC 255	21-Aug-08	29.194	364.14	2.20	165	3	2	0.08	8	135	0.6
GC 460	21-Aug-08	95.794	795.17	4.14	491	8	4	0.04	7	191	0.5
GC 763	21-Aug-08	230.713	1656.38	7.16	467	8	7	0.03	6	133	0.4
GC 1254	21-Aug-08	134.088	1874.56	8.30	657	11	8	0.06	7	141	0.4

Simultaneously extracted metals (SEM)
Acid volatile sulfides (AVS)
Total organic carbon (TOC)

Table 3-3. Physico-chemical readings during the 2008 Streamside Mesocosm exposure.

Streamside Mesocosm	Temperature (°C)	DO (mg/L)	Conductivity (uS/cm)	pH	DOC (mg/L)	Hardness (mg/L of CaCO3)	Alkalinity (mg/L of CaCO3)
Mean	23.22	6.79	701	7.91	3.4	290	252
St.dev	1.50	0.36	59	0.14	1.2	26	20

Dissolved Oxygen (DO)
Dissolved organic carbon (DOC)

Table 3-4. Sediment pH readings during the 2008 Streamside Mesocosm exposure. The pH readings were taken in the reference and high Ni treatment trays in both Greenville Creek (GC) and Big Beavercreek (BC) sediments. Surficial sediment (SS) measurements (< 2 cm) and deep sediment (DS) measurements (> 2 cm) were taken during the 28 d Ni-sediment exposure.

Treatment	Mean	St.dev
GC Reference SS		
Sediment pH	7.48	0.29
Sediment temp	23.1	1.4
GC Reference DS		
Sediment pH	7.36	0.23
Sediment temp	23.1	1.3
GC 1254 SS		
Sediment pH	7.06	0.40
Sediment temp	22.8	1.5
GC 1254 DS		
Sediment pH	6.65	0.11
Sediment temp	22.8	1.6
BC Reference SS		
Sediment pH	6.85	0.22
Sediment temp	23.0	1.4
BC Reference DS		
Sediment pH	6.82	0.18
Sediment temp	23.1	1.4
BC 7060 SS		
Sediment pH	6.51	0.19
Sediment temp	22.8	1.4
BC 7060 DS		
Sediment pH	6.32	0.27
Sediment temp	22.6	1.5

Table 3-5. Percent change for selected porewater sediment chemical variables during the 28 d Streamside mesocosm exposure. Percent change calculations were based on Day 0 (24-Jul-08), and calculated at 14 d (7-Aug-08) and 28 d (21-Aug-08).

Ni Treatment		Δ Total Ni	Δ SEM_{Ni}	Δ AVS	Δ Total Mn	Δ Total Fe	Δ TOC
Level	Date	(µmol/g)	(µmol/g)	(µmol/g)	(µmol/g)	(µmol/g)	(%)
BC Reference	7-Aug-08	-10	65	-5	0	34	9
BC 516	7-Aug-08	-25	-52	24	3	41	-13
BC 780	7-Aug-08	1	21	36	12	7	9
BC 1589	7-Aug-08	0	29	-13	4	0	-10
BC 3262	7-Aug-08	-15	-80	-16	8	0	-31
BC 7060	7-Aug-08	-23	-59	-28	25	0	-25
BC Reference	21-Aug-08	0	60	46	13	43	9
BC 516	21-Aug-08	-27	-15	55	4	17	18
BC 780	21-Aug-08	-29	59	52	18	7	10
BC 1589	21-Aug-08	-10	31	53	0	-12	-7
BC 3262	21-Aug-08	-31	-64	51	18	13	-10
BC 7060	21-Aug-08	-26	-34	46	21	-13	-24
GC Reference	7-Aug-08	0	52	-31	-10	-8	-11
GC 110	7-Aug-08	29	-20	25	1	27	10
GC 255	7-Aug-08	-6	-32	-16	13	19	6
GC 460	7-Aug-08	-3	-52	10	53	47	-34
GC 763	7-Aug-08	-38	-46	-19	-4	-3	-61
GC 1254	7-Aug-08	-50	-69	-28	33	53	-74
GC Reference	21-Aug-08	0	36	8	16	29	28
GC 110	21-Aug-08	15	-27	-24	7	27	19
GC 255	21-Aug-08	-35	-42	10	21	14	12
GC 460	21-Aug-08	7	-48	-47	11	79	-46
GC 763	21-Aug-08	-39	-47	-62	-4	7	-71
GC 1254	21-Aug-08	-48	-71	11	32	49	-82

Simultaneously extracted metals (SEM)
Acid volatile sulfides (AVS)
Total organic carbon (TOC)

Table 3-6. Multiple regression analyses from benthic macroinvertebrate responses in the Streamside Mesocosm for (SEM_{Ni}-AVS)/foc model.

Response	Coefficients	Value	T	P(>t)	R-sq	F	df	P	VIF < 4
Total Abundance	Intercept	85.4339	12.4	<0.0001	0.77	73.42	2, 45	<0.0001	0.000
	Total Fe	-0.0020	-5.2	<0.0001					2.050
	SEMNi-AVS/$f$oc	-8.2737	-11.4	<0.0001					2.050
Total Taxa	Intercept	3.1268	24.3	<0.0001	0.50	22.16	2, 45	<0.0001	0.000
	TOC	-0.5532	-6.6	<0.0001					1.937
	SEMNi-AVS/$f$oc	-0.0005	-5.3	<0.0001					1.937
Elmidae	Intercept	21.0698	4.2	0.0001	0.35	7.89	3, 44	0.0003	0.000
	Total Mn	-1.5907	-2.5	0.0167					1.154
	Total Fe	-1.0949	-4.6	<0.0001					2.301
	SEMNi-AVS/$f$oc	-0.0003	-2.3	0.0292					2.083

Table 3-7. Multiple regression analyses from benthic macroinvertebrate responses in the Streamside Mesocosm for SEM_{Ni}-AVS model.

Response	Coefficients	Value	T	P(>t)	R-sq	F	df	P	VIF < 4
No of Ephemeroptera Taxa	Intercept	1.8101	23.6	<0.0001	0.71	55.15	2, 45	<0.0001	0.000
	SEMNi	-0.3523	-10.3	<0.0001					1.016
	SEMNi-AVS	0.0073	3.1	0.0030					1.016
No of EPT Taxa	Intercept	1.8516	21.4	<0.0001	0.66	43.78	2, 45	<0.0001	0.000
	SEM-Ni	-0.3539	-9.2	<0.0001					1.016
	SEMNi-AVS	0.0072	2.8	0.0081					1.016

Table 3-8. Multiple regression analyses from benthic macroinvertebrate responses in the Streamside Mesocosm for SEM_{Ni}/AVS model.

Response	Coefficients	Value	T	P(>t)	R-sq	F	df	P	VIF < 4
Caenidae	Intercept	5.6583	16.0	<0.0001	0.78	79.98	2, 45	<0.0001	0.00
	AVS	-0.9514	-10.8	<0.0001					3.91
	SEMNi/AVS	-1.2645	-12.7	<0.0001					3.91
% Ephemeroptera Taxa	Intercept	37.4527	12.04	<0.0001	0.70	51.51	2, 45	<0.0001	0.00
	SEMNi/AVS	-8.8978	-10.11	<0.0001					3.90
	Total Ni	-7.0664	-9.15	<0.0001					3.90
% EPT Taxa	Intercept	37.1010	2330.19	<0.0001	0.64	39.57	2, 45	<0.0001	0.00
	SEMNi/AVS	-6.8786	-9.32	<0.0001					3.90
	AVS	-8.6044	-9.57	<0.0001					3.90
Heptageniidae	Intercept	12.1675	12.4	<0.0001	0.63	38.33	2, 45	<0.0001	0.00
	SEMNi/AVS	-0.2211	-5.2	<0.0001					2.93
	Total Fe	-1.1563	-11.4	<0.0001					2.93
Coenagrionidae	Intercept	2.0126	7.6	<0.0001	0.61	23.18	3, 44	<0.0001	0.00
	Date	0.4033	3.7	0.0007					1.01
	SEMNi/AVS	-0.3303	-5.9	<0.0001					3.64
	AVS	-0.4975	-7.7	<0.0001					3.66
Ephemeridae	Intercept	1.4372	8.7	<0.0001	0.56	18.64	3, 44	<0.0001	0.00
	Date	0.2242	3.2	0.0024					1.02
	SEMNi/AVS	-0.2065	-5.8	<0.0001					3.96
	AVS	-0.2868	-7.1	<0.0001					3.98
No of Burrower Taxa	Intercept	-1.0429	-3.0	0.0063	0.54	26.83	2, 45	<0.0001	0.00
	Substrate	0.8234	7.3	<0.0001					3.83
	SEMNi/AVS	-0.1749	-6.0	<0.0001					3.83
% Top Dominant	Intercept	41.7840	7.4	<0.0001	0.40	15.04	2, 45	<0.0001	0.00
	SEMNi/AVS	6.2332	5.03	<0.0001					3.46
	TOC	7.9063	3.06	0.0037					3.46
% Chironomidae	Intercept	41.7840	7.4	<0.0001	0.40	15.04	2, 45	<0.0001	0.00
	SEMNi/AVS	6.2332	5.03	<0.0001					3.46
	TOC	7.9063	3.06	0.0037					3.46
Shannon Diversity	Intercept	7.8191	10.61	<0.0001	0.40	15.21	2, 45	<0.0001	0.00
	SEMNi/AVS	-0.8587	-5.31	<0.0001					3.46
	TOC	-1.2378	-3.68	0.0006					3.46
Hills Diversity Number N1	Intercept	12.1834	13.89	<0.0001	0.39	14.09	2, 45	<0.0001	0.00
	SEMNi/AVS	-0.9979	-5.18	<0.0001					3.46
	TOC	-1.5060	-3.76	0.0005					3.46
Simpsons Diversity Index	Intercept	3.1026	7.77	<0.0001	0.38	13.67	2, 45	<0.0001	0.00
	SEMNi/AVS	0.4167	4.75	<0.0001					3.46
	TOC	0.5187	2.84	0.0067					3.46
Ceratopogonidae	Intercept	-1.7739	-3.4	0.0016	0.38	13.78	2, 45	<0.0001	0.00
	Substrate	0.9017	5.3	<0.0001					3.58
	SEMNi/AVS	-0.2000	-4.5	<0.0001					3.58
% Sprawlers	Intercept	39.6210	7.48	<0.0001	0.37	13.24	2, 45	0.0003	0.00
	SEMNi/AVS	-5.9305	-5.1	<0.0001					3.46
	TOC	-9.5478	-3.95	0.0003					3.46
% Scrappers	Intercept	107.7101	5.12	<0.0001	0.37	13.1	2, 45	<0.0001	0.00
	SEMNi/AVS	-1.6613	-3.44	0.0013					2.92
	Total Fe	-10.7993	-5.01	<0.0001					2.92
% Burrowers	Intercept	43.9984	7.31	<0.0001	0.35	12.11	2, 45	<0.0001	0.00
	SEMNi/AVS	6.0440	4.55	<0.0001					3.46
	TOC	7.7579	2.82	0.0071					3.46
Margarlef Richness	Intercept	9.6094	11.83	<0.0001	0.34	11.51	2, 45	<0.0001	0.00
	SEMNi/AVS	-0.8285	-4.65	<0.0001					3.46
	TOC	-1.6930	-4.56	<0.0001					3.46
Hills Diversity Number N2	Intercept	10.0353	11.91	<0.0001	0.30	9.79	2, 45	0.0003	0.00
	SEMNi/AVS	-0.7365	-3.98	0.0002					3.46
	TOC	-0.8952	-2.33	0.0246					3.46

Table 3-9. Multiple regression analyses from benthic macroinvertebrate responses in the Streamside Mesocosm for Total Ni.

Response	Coefficients	Value	T	P(>t)	R-sq	F	df	P	VIF < 4
Chironomidae	Intercept	49.1118	9.37	<0.0001	0.71	55.98	2, 45	<0.0001	0.00
	Total Ni	-0.6528	-3.17	0.0027					1.26
	Total Fe	-4.3732	-7.57	<0.0001					1.26
Hills Ratio E1	Intercept	6.0959	9.22	<0.0001	0.53	25.61	2, 45	0.0028	0.00
	Total Ni	0.1808	2.66	0.0107					1.26
	Substrate	-0.7721	-4.69	<0.0001					1.26

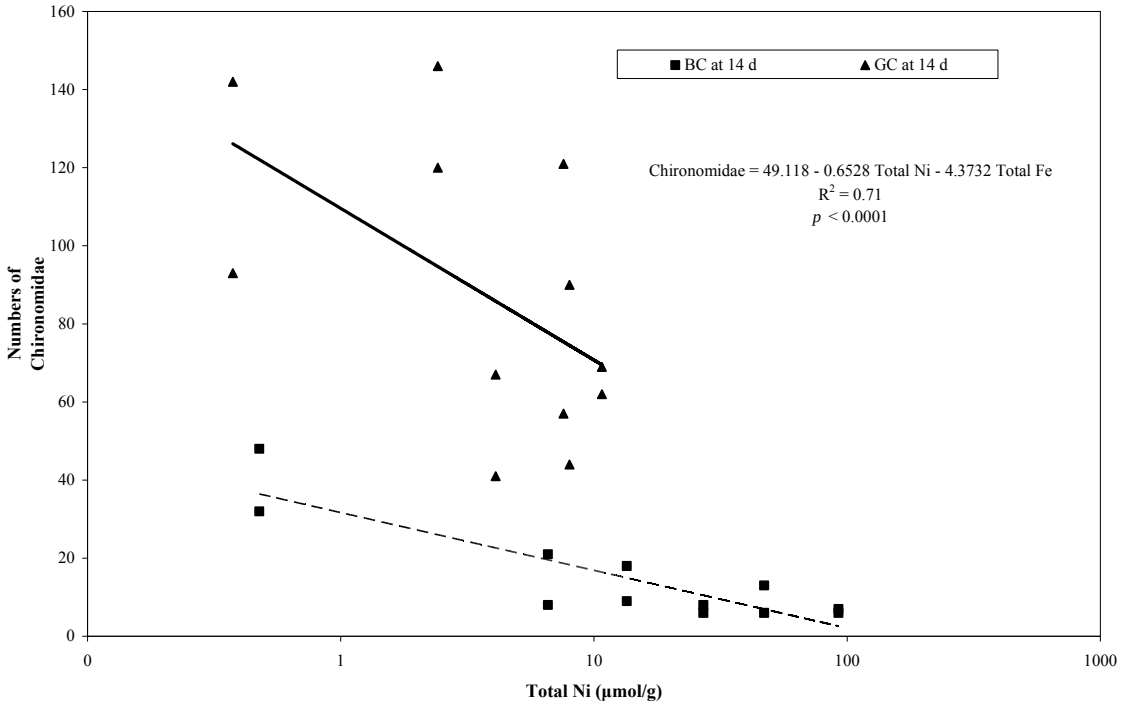

Figure 3-1. Chironomidae responses were negatively affected by increasing log Ni concentrations at 14 d in both GC and BC sediments. Dark regression line is BC 14 d, and dashed regression line is GC 14 d.

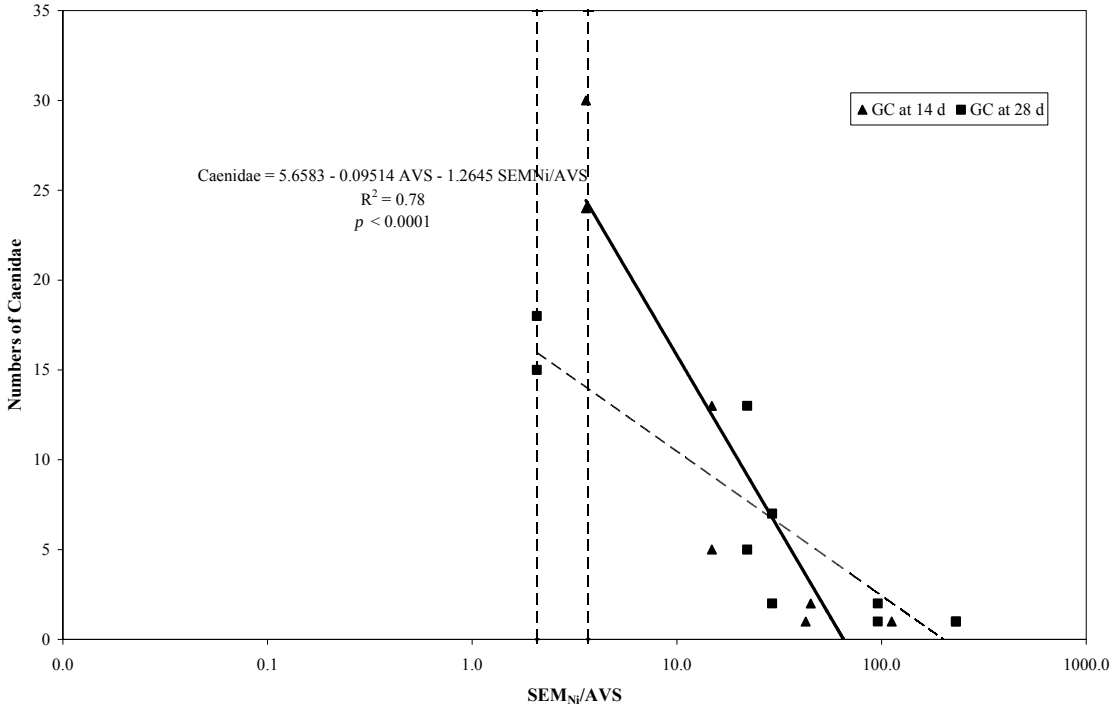

Figure 3-2. Caenidae numbers declined with increasing log SEM_{Ni}/AVS in GC sediments at 14 and 28 d. Dashed lines represent SEM_{Ni}/AVS 8 and 40, range of uncertainty. Dark regression line is GC 14 d, and dashed regression line is GC 28 d.

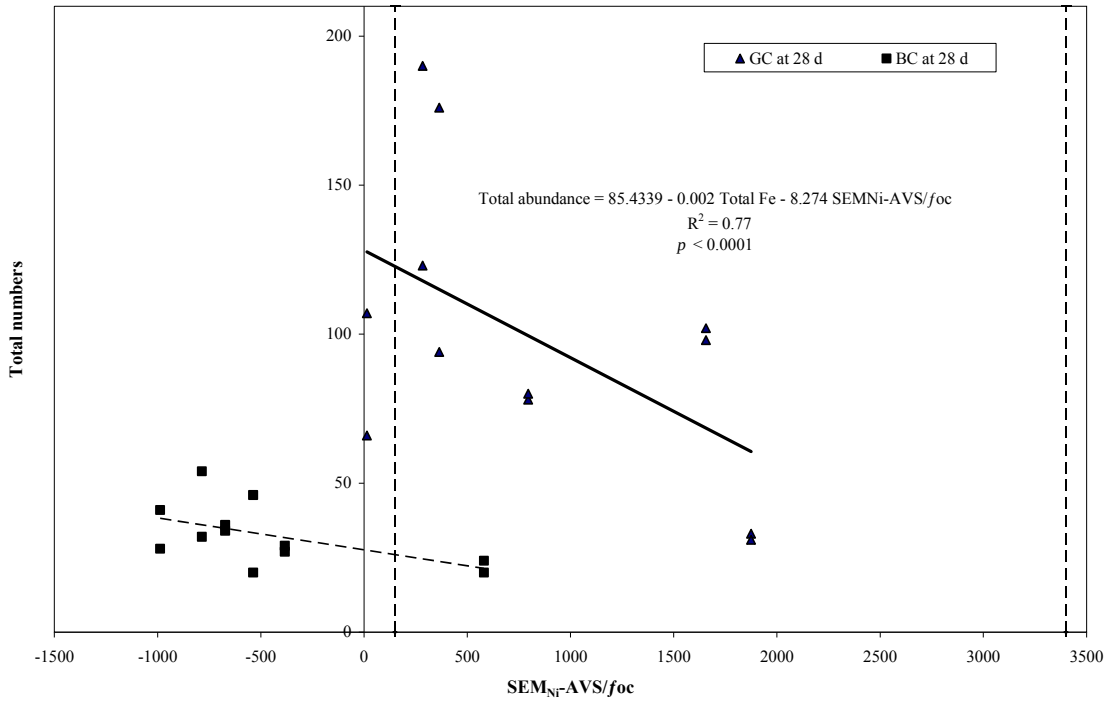

Figure 3-3. Total abundance decreased with increasing SEM_{Ni}-AVS/*foc*, and total abundance of macroinvertebrates was higher in GC sediments vs. BC sediments. Dashed lines represent (SEM-AVS)/*foc* 150 and 3400, range of uncertainty. Dark regression line is GC 28 d, and dashed regression line is BC 28 d.

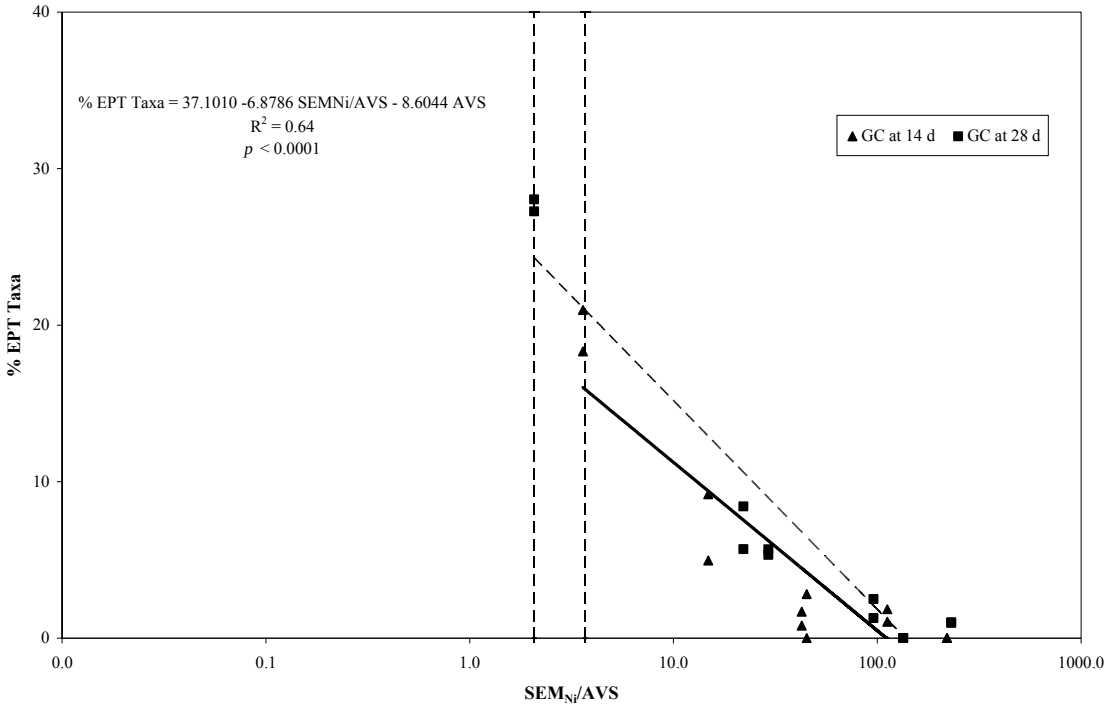

Figure 3-4. The % EPT Taxa declined with increasing SEM_{Ni}/AVS on GC sediments at both dates (14 and 28 d). Dashed lines represent SEM_{Ni}/AVS 8 and 40, range of uncertainty. Dark regression line is GC 14 d, and dashed regression line is GC 28 d.

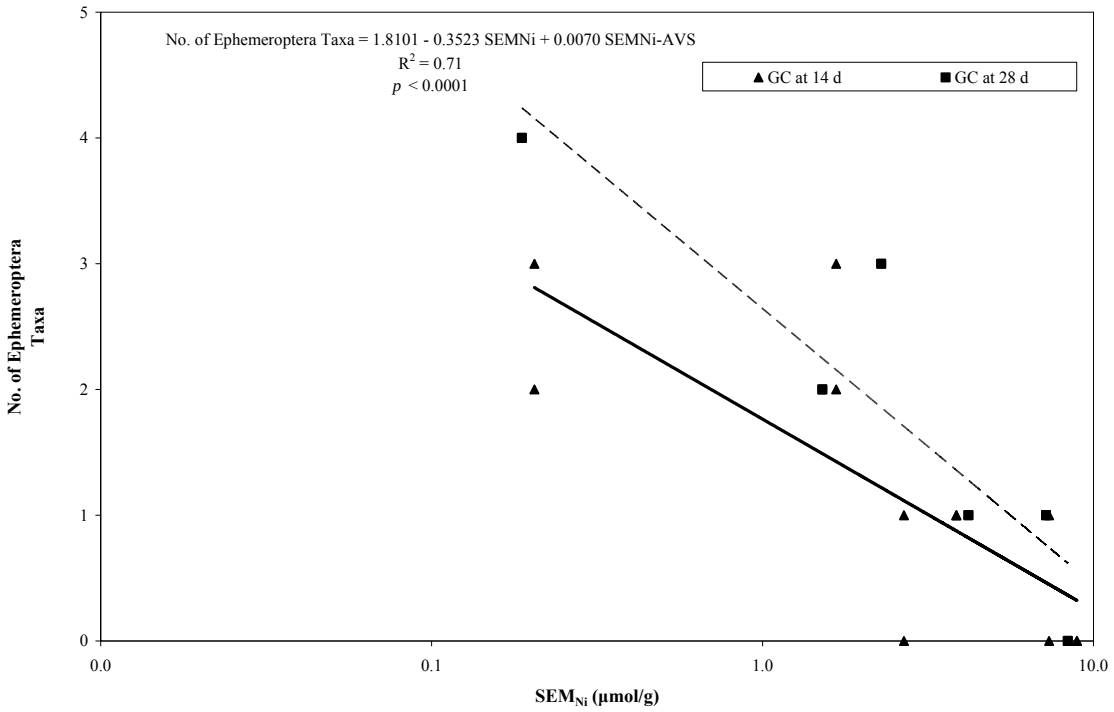

Figure 3-5. The number of Ephemeroptera taxa decreased with increasing SEM_{Ni} on GC sediments at both dates (14 and 28 d). Dark regression line is GC 14 d, and dashed regression line is GC 28 d.

CHAPTER 4 – INDIGENOUS AND SURROGATE ORGANISM RESPONSES TO NICKEL SEDIMENT EXPOSURES IN FLOW-THRU TESTS (2008-2009)

1-0 ABSTRACT

Single species Ni sediment toxicity was examined in laboratory flow-thru exposures. Indigenous mayfly (*Anthopotamus verticis, Isonychia spp.*, and *Stenonema spp.*), beetle (*Psephenus herricki*) larvae, and surrogate species (*Hyalella azteca* and *Chironomus dilutus*) were exposed to a serial series of Ni amended sediment in 7 d and 10 d tests, respectively. Sediments (low in acid volatile sulfides (AVS) and organic carbon (TOC)) were used to examine Ni toxicity. Unfiltered well water was diluted with deionized water to a hardness of 180 - 200 mg/L of $CaCO_3$. *Hyalella azteca* was the most sensitive species tested in this study, with survival LC_{10} of 0.4 µmol/g and dry weight IC_{25} of 0.6 µmol/g. *Anthopotamus verticis* proved to be the most sensitive indigenous species tested. All the organisms tested showed reduced survival with elevated Ni exposures, with the exception of *C. dilutus* and *P. herricki*. *Isonychia spp.* survival demonstrated a significant survival effect, and *P. herricki* did not respond to the highest Ni concentration tested (112 µmol/g). *Anthopotamus verticis* and *Stenonema spp.* showed similar sublethal sensitivities to Ni with IC_{25} values for lengths, AFDW and dry weights, and head capsule widths having overlapping 95% CI.

Anthopotamus verticis and *Stenonema spp.* had moderate survival sensitivity to Ni compared to *H. azteca*. An alternative to the USEPA acid volatile sulfide (AVS) and

simultaneously extracted metal (SEM_{Ni}) methodology was tested on three sediment types to determine if treatments varied. There was no statistical difference between extracted SEM_{Ni} in low AVS and moderate/high AVS sediments. However, there was no difference in high AVS sediments. Ni toxicity was apparent with indigenous and surrogate organisms in lab flow-thru conditions, and demonstrating both lethal and sublethal responses.

2-0 INTRODUCTION

Metals in the environment can have detrimental effects on humans and biota. Metals that enter aquatic systems can reside in sediments for long periods of time due to sequestration by solid phases. These solid phases are complex, and without an understanding of how metals partition and become bioavailable the potential toxicity of sediments could be missed. Sources of Ni in the environment include Ni mining, Ni manufacturing, solid waste incinerators, oil and coal combustion (Sen Gupta and Bhattacharyya 2008).

USEPA surrogate organisms (*Daphnia magna, Ceriodaphnia dubia, Hyalella azteca, Chironomus dilutus,* and *Pimephales promelas*) are commonly used to evaluate toxicity to many contaminants in both laboratory and *in situ* toxicity tests (USEPA 2000; Burton et al. 2005a). There is a need to determine whether USEPA surrogate organisms are protective of indigenous organisms (e.g. *Isonychia spp., Stenonema spp.*, *Anthopotamus verticis*, and *Psephenus herricki)* in Ni sediments tests. Early instars or

life stages of surrogate organisms are commonly used for testing purposes, but capturing these life stages of indigenous organisms is difficult (Diamond et al. 1990; Irving et al. 2003; Custer et al. 2006). Federal and state ambient water quality standards are designed to protect 95% of the resident species and more specifically, threatened and endangered species (ES) (USEPA 1999). However, because ES listed species appear to be more sensitive, the 95% guideline may not protect all Endangered Species Act listed species (USEPA 1999).

Understanding how Ni affects later instars of indigenous organisms is warranted because early instars of these organisms may not be present at all times when collecting (i.e. emergence, and overwintering). It is commonly accepted that the early life stage of an organism is the most sensitive. If these early life stages of indigenous species cannot be tested, the best way is to compare later instar insect responses to the USEPA surrogates responses, and these can be carried out *in situ* or in a laboratory exposure. Recently, Echols et al. (2010) demonstrated that later instar *Isonychia bicolor* were more sensitive to coal mine effluent than *C. dubia*. These findings are promising for using indigenous aquatic insects in both laboratory and field toxicity testing.

2-1 Objective - The objectives of this study were to compare Ni sensitivities of four indigenous aquatic insects, and two USEPA surrogate organism responses (lethal and sublethal) to Ni-spiked sediments in flow-thru exposures. Also, an abbreviated method

for simultaneously extracted metal (SEM_{Ni}) was compared to the standard USEPA SEM/AVS method.

2-2 Hypothesis: *Isonychia spp.* < *P. herricki* < *Stenonema spp.* < *A. verticis* in Ni sensitivity, and *Isonychia spp.* growth will be most sensitive sublethal endpoint for all indigenous insects.

3-0 MATERIALS & METHODS

3-1 Laboratory design

Tests were performed in a laboratory flow-through design which used a blend of unfiltered well water and deionized water to a desired hardness of ~200 mg/L of $CaCO_3$. The flow-through design used traditional Zumwalt water delivery for sediment toxicity tests (USEPA 2000), and was modified to receive water flow continuously for 7-10 d (Fig 4-1). A series of three 757 L carboys were used; first one received raw well water and deionized water (DI) for desired hardness, and settling of solids. The water flowed to the second and third carboys which were aerated continuously. The third carboy was pumped through a parallel series of sediment filter, then a carbon filter, and then flowed to the flow-thru system.

3-2 Sediment collection, spiking, and deployment

Nickel concentrations for all tests used a dilution series with a 0.5 dilution factor, and each test had five Ni concentrations plus a reference. Each concentration had four

replicates plus an additional replicates for and physico-chemical monitoring (DO, Conductivity, sediment and water temperature, and pH). All sediments were spiked with Ni as described in Chapter 2, sec 3-3. Sediments added were ~100 ml, with ~175 ml overlying water. Ni-spiked sediments were added and flushed for 2-3 hrs prior to any organisms being added.

3-3 Physico-chemical and sediment pH monitoring

Dissolved oxygen (DO), conductivity, temperature, pH were measured daily with YSI-85 and YSI-pH100 meters. Sediment pH, sediment temperature was measured 3 times during each test (every other day), and hardness/alkalinity were measured at the beginning and end of the test. Dissolved organic carbon (DOC), and overlying water Ni samples were taken on Days 1 and 7. All DOC and Ni samples were collected with acid-clean 50 ml syringes, and 0.45 μm syringe filters. Samples were analyzed as described in Chapter 3, sec 3-5. Flow rates entering the beakers were estimated by capturing 40 ml of water from the Zumwalt needles, and timing until desired volume was reached. Flow rates were performed in replicates, and calculated on basis of volume/time.

3-4 Benthic invertebrate collection, transport, and culturing

The mayflies *(Anthopotamus verticis, Isonychia spp.*, *Stenonema spp.)* and the beetle (*Psephenus herricki)* were field collected from the Great Miami River and Greenville Creek, OH, USA. Collection techniques included using a kick seine (1588

μm), or D-frame dipnet (794 μm). Organisms were collected with regard to uniform size, and forceps were used to transfer organisms to coolers equipped with air pumps. Substrate (leaves and tree branches) were placed in the coolers and organisms were transported to the lab within 8 hr.

Chironomus dilutus and *Hyalella azteca* were cultured in the laboratory and followed USEPA (2000). *Chironomus dilutus* were < 10 d old (2^{nd} – 3^{rd} instar) and *H. azteca* were between 7-14 d old when beginning a test. These organisms were cultured with a blend of unfiltered well water and deionized water. *Chironomus dilutus* were fed Tetramin slurry, and *H. azteca* were fed ground rabbit food daily. All organisms were cultured and tested under 16:8 h light/dark cycle, and controlled temperatures (20-22°C).

Ten organisms were loaded into Ni-amended beakers after overlying water had been flushed of Ni (2-3 hrs), and each beaker was fed daily. *Isonychia spp*. larvae were fed 0.5 ml of blended stream conditioned sycamore and maple leaves that were field collected. *Anthopotamus verticis*, *Stenonema spp*., and *P. herricki* were fed 1.0 ml of algae (*Selenastrum capricornutum*). *Hyalella azteca* were fed ~1 mg of ground rabbit pellets, and *C. dilutus* fed 1 ml of Tetramin slurry.

3-5 Organism AFDW, dry weight, length, head capsule width, and exuvia

When sediment toxicity tests were terminated, survival and other sublethal endpoints (Dry weight, Ash-free dry weights (AFDW), head capsule widths, Exuvia) were measured. All pans were conditioned by ashing aluminum weigh pans at 500°C for

1 h, and then weighed to the nearest 0.01 mg. All surviving organisms from each replicate were added, and dried at 100 ± 5°C for 24 ± 2 h. If AFDW were used, dry weights recorded and organisms placed in muffle furnace at 500°C for 1 h (Benke et al. 1999), and weighed to the nearest 0.01 mg. Lengths and head capsule widths were measured to the nearest 1mm using a transparent ruler or an ocular micrometer on a stereomicroscope. Exuvia counts were collected daily, and removed from the replicate. The mayflies and beetle tests did not require sieving sediments to recover organisms, but *C. dilutus* and *H. azteca* almost always were required.

3-6 Sediment sampling and sediment chemical characterization

The sediment chemical characterization (TOC, AVS, and Total Metals) followed the methods and analyses as described in Chapter 2, sec. 3-6 and 3-7. All sediment chemical concentrations are presented as concentration on a dry weight basis.

3-7 SEM_{Ni} from the abbreviated AVS/SEM method

An alternative to the full SEM/AVS method (USEPA 2005) was developed for extracting SEM_{Ni} from sediments which have low concentrations AVS (< 0.1 μmol/g of H_2S). A comparison of SEM_{Ni} concentrations from the full method and the abbreviated method was used.

The abbreviated method consisted of using 250 ml acid-cleaned beakers with 100 ml of deoxygenated DI water. Approximately 10 g of GC sediments, 2 g of BC

sediments, and 1 g of WD sediments were wrapped in parafilm, added to the beakers with DI water, and shaken at an RPM of 180 for 7 min. After 7 min, the beakers were slowed to an RPM < 20, and 20 ml of 6 M HCl was added. The RPM was turned up to 180 for 50 min. After sediments were finished shaking, each beaker was rinsed with DI water and filtered thru an acid-cleaned 0.45 μm membrane filter. The filtrate was then brought a final volume of 250 ml, and 50 ml aliquot was transferred to an acid-cleaned 50 ml centrifuge tube for storage until analyses was performed. All acid additions to the beakers were conducted under a fume hood.

The other replicates of GC, BC, and WD sediments were analyzed using the USEPA (2005) full method, and comparisons were made by statistically testing just SEM_{Ni} concentrations from both methods. If SEM_{Ni} concentrations were not statistically different, then the appropriate AVS concentrations were assumed and reported for the appropriate reference and Ni-amended sediment replicates (Tables 4-1, 4-2). Aliquot of SEM_{Ni} dilutions were placed in 15 ml acid-cleaned centrifuge tubes. All analyses were performed on Perkin Elmer Flame AA, with blanks and standards used in the standard curve and QA/QC.

AVS was only analyzed on the *A. verticis* Day 0 and Day 7 samples using the USEPA (2005) method, and all values reported in AVS μmol/g dry weight. The other three indigenous tests (Stenonema spp., *Isonychia spp*., *P. herricki*) and two surrogate species tests (*H. azteca*, *C. dilutus*) were assigned assumed AVS values. All assumed AVS values for these remaining Ni flow-thru tests were calculated as the mean between

Day 0 and Day 7 values for the respective treatment from the *A. verticis* test. It is understood that this is an assumption, and provides only an estimate when using these values in the SEM_{Ni}/AVS models. These GC sediments have low AVS content, and varied little in AVS during recent studies.

AVS samples were analyzed on a Thermofisher Spectrophotometer, and QA/QC samples consisted of blanks, blank spikes, sample spikes, and duplicates. Standard curves were generated for low sulfide concentrations (µmol/ml), and reported as µmol/g dry weight.

3-8 Data Analysis

All survival and sublethal LC_{10} and IC_{25} results were calculated on Toxcalc 5.0. Regression equations, two-sample *t*-tests, and One-way ANOVA results were generated on Minitab 16. SEM_{Ni} extraction comparisons were tested using a two-sample *t*-test. Tukey's pairwise comparisons were made for all One-way ANOVA results that were significant. All assumptions were tested for regression, *t*-tests, ANOVA analyses, and data was transformed or non-parametric analyses were performed when necessary. All LC_{10} and IC_{25} results are presented as the point estimate (95% confidence interval). Survival data is presented at mean % survival ± standard deviation, and sublethal data (dry wt, AFDW, length, head capsule, exuvia) presented at mean ± standard deviation.

4-0 RESULTS AND DISCUSSION

4-1 Sediment chemistry and bioavailability

GC sediments were used in all tests, and these sediments are low in AVS and TOC (Tables 4-1, 4-2). There were similarities in total Fe, total Mn, and TOC in all tests (Tables 4-1, 4-2), and AVS was estimated for all tests except *A. verticis* test (Tables 4-1, 4-2). The range of Total Fe for the indigenous tests was 86-161 μmol/g, and the surrogate tests were 86-522 μmol/g (Tables 4-1, 4-2). The total Mn range for the indigenous tests was 5-9 μmol/g, and 4-9 μmol/g for the surrogate tests. TOC ranged from 0.4-3.4 % in the indigenous tests, and 0.1-1.2% for the surrogate test (Tables 4-1, 4-2).

The GC sediment chemistry characteristics were similar to those observed in Chapter 3, Table 3-2, which showed low AVS, TOC, Fe, and moderate Mn. TOC content was low, and TOC increased with increasing Ni in Day 0 samples, this trend was also observed in Chapter 3 (Table 3-2). This increase in TOC is probably a function of excess Ni and the LOI methodology. This pattern was examined further, and a split sample of a high Ni GC sample was analyzed by the LOI method (Heiri et al. 2001) and at Alloway Labs with a carbon analyzer. The GC sample through the LOI method had a % TOC of 2.04, and result from Alloway was < 0.1 %. If the carbon analyzer results are correct, this suggests the LOI may be inflating % TOC values in the high Ni treatments. Since these sediments have low TOC content, and it is not likely that TOC is increasing with increasing Ni, the implications from using the $(SEM_{Ni}\text{-}AVS)/foc$ model is that the

amount of bioavailable Ni may be underestimated, when it is highly likely that there is less OC available.

With these high Ni concentrations in the indigenous tests, Ni was fluxing from the sediments. Ni flux was only monitored in three tests (one indigenous and both surrogate tests) (Table 4-3). From previous studies (Chapter 2 and 3), it has been shown that Ni is fluxing from sediments during short-term and long-term tests, especially GC type sediments. Ni flux assumptions were made for the *P. herricki*, *Stenonema spp.*, and *Isonychia spp*. tests which were based on *A. verticis* test data (Table 4-3). Ni loss from *A. verticis* test was showing that a greater percentage was being lost as the Ni concentration increased (Table 4-3). As much as 76 and 71% was lost in the two highest Ni treatments after 7 d (Table 4-3). Contrasting to 35 and 23% for *C. dilutus*, and 61 and 0% for *H. azteca*.

The assumption that Ni flux was occurring in the three remaining indigenous tests were made based on previous studies (Chapters 2 and 3), and supporting data (Tables 2-1c, 3-5). Ni flux from sediments (from Day 0 to Day 7) was consistent with results from previous two studies (Chapters 2 and 3). The more Ni being added to the sediments (i.e. high Ni treatments), the more Ni loss is being observed (Table 4-3). As seen in other studies (Chapter 2 and 3) this type of sediment (GC) has higher bioavailable Ni, and the most Ni flux. Costello et al. (2011) have developed Ni spiking method that requires a longer equilibration time (> 30 d), and adjusting pH over time. Liber et al. (2011) has stated that there is no consensus among scientists regarding equilibration times for Ni-

spiked sediment tests. In the current study, equilibration times were 1-3 d, and this was followed throughout the studies in the dissertation to facilitate comparisons to each individual study.

4-2 Physico-chem and sediment pH

Flow rates in the flow-thru design were estimated by collecting water exiting the Zumwalt needles. The flow-rate was estimated at 190.5 ± 16.5 ml/min for each beaker. Sediment pH declined with increasing Ni concentration in all tests, and sediment temperature was consistent throughout all tests (Table 4-4). Overlying water temperature, conductivity, pH, hardness and alkalinity were very similar in all tests (Table 4-4). In each test the hardness ranged from 180-197 mg/L of $CaCO_3$, and alkalinity ranged from 158-191 mg/L of $CaCO_3$ (Table 4-4). Temperature ranged from 20.3 – 22.2 °C, DO 7.07 – 8.46 mg/L, conductivity from 366-442 µS/cm, and pH from 7.76 – 7.94.

Overlying water was being exchanged a moderate rate (11.4 ± 1.0 L/h) throughout the study, and provided adequate dissolved oxygen (DO) in the beakers. The blend of DI and well water was held constant throughout all tests, and physico-chem parameters reflected similar levels in all tests. Sediment pH declines with increasing Ni concentration were consistent with previous studies (Chapters 2 and 3). Authors have suggested that increased pH and Eh values in the surficial sediments may be a function of this layer becoming more oxic from bioturbation (Goldhaber 2003, De Jonge et al. 2012).

4-3 SEM_{Ni} *extraction method*

The GC sediments had low AVS content and an alternative method was used to extract SEM_{Ni} using an orbital shaker and beakers. The three replicates of WD, BC, and GC were analyzed for SEM_{Ni} using the above method and the full USEPA AVS method. There were no statistical differences ($p > 0.05$) detected between the two different methods on any of the sediment types (Table 4-5). WD sediment had a marginal *p*-value of 0.07, but the GC sediments *p*-value was 0.328 (Table 4-5). SEM_{Ni} for all tests except *A. verticis* were analyzed with the shaker method (Tables 4-1, 4-2). The SEM_{Ni} and AVS concentrations for both *A. verticis* Day 0 and Day 7 were analyzed with the USEPA AVS method. The AVS concentrations from the averaged Day 0 and Day 7 samples were used for all the remaining sediment chemistry samples and calculations (Tables 4-1, 4-2).

The abbreviated SEM_{Ni} extraction appears to be a practical method for extracting SEM_{Ni} from sediments with low AVS content. Numerous samples (15-20) can be extracted in a given hour versus one sample/hour with the full SEM/AVS method. However, given the marginally statistical result ($p = 0.07$) in WD sediments, this may warrant further testing when using the abbreviated method with high AVS sediments (BC and WD). Assumptions of AVS in anoxic sediments (e.g. WD and BC) may not be practical given the spatial and seasonal differences in AVS content within sample matrix of anoxic sediments samples (Rickard and Morse 2005). It is recommended to use this method after sediments with low AVS have been identified, and run with the full method

to determine accurate AVS concentrations. These oxic type sediments (GC and MR) appear have consistent low AVS values, and this abbreviated method could allow for higher volume of samples to be characterized.

4-4 Biological Responses

Threshold effect levels and endpoint analyses

The indigenous and surrogate test organisms responded to Ni differently, and apparent divergent sensitivities were observed (Table 4-6). Indigenous insect Ni threshold effect levels varied, and demonstrated that mayfly burrowers (*A. verticis*) were most sensitive (LC_{10} and IC_{25}) (Table 4-6). All Ni threshold effect levels results were based on Day 0, unless otherwise noted.

Anthopotamus verticis survival response (LC_{10} 3.2 (1.4, 5.1) µmol/g) was the most sensitive endpoint of the four insects in lab flow-through exposures (Table 4-6). *Anthopotamus verticis* sensitivity to Ni-spiked sediments in the flow-thru was ranked as, survival < dry weight < AFDW < lengths = head capsule widths (Table 4-6, Figs 4-2, 4-3). *Stenonema spp.* sensitivity was similar to *A. verticis* and ranked as survival < dry weight < AFDW < head capsule widths < lengths (Table 4-6, Figs 4-4, 4-5). *Stenonema spp.* survival response (LC_{10} 6.0 (3.2, 8.8) µmol/g) was the most sensitive endpoint during the Ni-sediment flow-thru test. *Isonychia spp*. sensitivity to Ni-spiked sediments was ranked as exuvia < Survival < length = Dry weight = head capsule width = AFDW (Table 4-6). The *Isonychia spp*. exuvia threshold level was (IC_{25}) 19.0 (no CI) µmol/g,

and the survival threshold level was 19.8 (7.1, 35.6) µmol/g (Table 4-6). An *Isonychia spp*. survival effect was observed, but only at the highest Ni concentration (Fig 4-6).

The overall LC_{10} survival sensitivity ranking for the indigenous insects from most sensitive to least sensitive was, *A. verticis* < *Stenonema spp.* < *Isonychia spp.* < *Psephenus herricki* (Table 4-6, Figs 4-2 – 4-6). The IC_{25} dry mass sensitivity for the indigenous insects ranked in order from most sensitive to least sensitive: *A. verticis* < *Stenonema spp.* < *Isonychia spp.* < *P. herricki* (Table 4-6, Figs 4-3, 4-5). The IC_{25} AFDW sensitivity for the indigenous insects ranked in order from most sensitive to least sensitive: *Stenonema spp.* < *A. verticis* < *Isonychia spp.* < *P. herricki* (Table 4-6, Figs 4-3, 4-5).

During the Ni flow-thru tests *H. azteca* was more sensitive than *C. dilutus* in both LC_{10} survival and IC_{25} dry weight (Table 4-6). The *H. azteca* LC_{10} was 0.4 (0, 1.0) µmol/g and *C. dilutus* LC_{10} was 32.3 (no CI) (Table 4-6, Fig. 4-7). The *H. azteca* IC_{25} was 0.6 (0.3, 1.8) µmol/g and *C. dilutus* IC_{25} was 2.2 (1.0, 4.9) µmol/g (Table 4-6). Dry weight < AFDW < Survival were the most sensitive endpoints for *C. dilutus* (Table 4-6). *Hyalella azteca* Day 0 and Day 10 LC_{10} values increased from Day 0 Ni concentrations (lower) to Day 10 Ni concentrations (higher), whereas, *C. dilutus* values decreased (Table 4-6). *Chironomus dilutus* Day 0 and Day 10 sublethal threshold effect levels were both lower and Ni concentrations decreased from Day 0 to Day 10. However, *H. azteca* had increased IC_{25} values for dry weight from Day 0 to Day 10 (Table 4-6).

Anthopotamus verticis showed Ni-sediment effects ($p < 0.05$) with survival, lengths, dry weight, and AFDW (Table 4-7). *Stenonema spp.* Ni-sediment effects were detected in survival, head capsule widths, dry weight, AFDW, and exuvia ($p < 0.05$) (Table 4-7). *Isonychia spp.* showed survival effects ($p < 0.05$) in the highest Ni concentration (Table 4-7). *Chironomus dilutus* dry weight and AFDW were statistically significant ($p < 0.05$) (Table 4-7). *Hyalella azteca* showed significant survival effects ($p < 0.05$), but dry weights were not showing effects from the Ni sediment tests (Table 4-7).

In this study, *A. verticis* demonstrated moderate sensitivity to Ni when compared to *H. azteca* and *Isonychia spp*. Field collected *Isonychia bicolor* were as sensitive as *C. dubia* (Echols et al. 2010), and *H. azteca* is a known sensitive organism to Ni sediment tests (USEPA 2000, Doig and Liber 2006, Liber et al. 2011). In this study, *A. verticis* LC_{10} survival was ~ 6x more sensitive than *Isonychia spp. Anthopotamus verticis* and *Stenonema spp.* were the most sensitive indigenous organisms tested; however, *A. verticis* survival, dry weight, and AFDW values were all lower than *Stenonema spp.* (Table 4-6).

Anthopotamus verticis increased Ni sensitivity may reside in its detritus filter-feeding habits (Bae and McCafferty 1991). *Anthopotamus verticis* are filtering fine particulate organic matter (FPOM) that has deposited in the sediments. Bae and McCafferty (1991) examined gut contents of *A. verticis* and found < 5% diatoms. Since OM is an important ligand for Ni complexation (Di Toro et al. 2005), dietary route of exposure may be specifically important to *A. verticis*. Kiffney and Clements (1994,

1996) found diet was contributing to Heptageniidae metal sensitivity. This study suggests that *Stenonema spp*. (family Heptageniidae) were sensitive to Ni, but contrary to the other scraper tested, *P. herricki*. *Psephenus herricki* did not respond to the highest Ni concentration (112.7 µmol/g). Thus, suggesting the *P. herricki* is tolerant of Ni (Table 4-7). Custer et al. (2006) found *P. herricki* responded to ammonia during *in situ* toxicity tests. This species is not a traditionally test organism, however in the presence of Ni, *P. herricki* was not sensitive to Ni.

Chironomus dilutus dry weight sensitivity (IC_{25} 2.2 µmol/g) was more sensitive than its AFDW (IC_{25} 4.7 µmol/g). This may suggest that dry weights are a sufficient endpoint in Ni spiked toxicity tests when using GC type sediments (low AVS and TOC). The USEPA (2000) suggests AFDW is more sensitive endpoint than survival for *C. dilutus,* and AFDW is recommended because of sediment particle ingestion possibility.

H. azteca was overall, the most sensitive to Ni in the experiment; however the exposure times (10 d) were different for *H. azteca* and *C. dilutus*, than the indigenous insects (7 d). The rationale behind the different exposures times was two-fold. First, *H. azteca* and *C. dilutus* tests were 10 d to generate threshold values from a flow-thru test versus traditional static-renewal tests. Second, there was concern surrounding bringing field collected indigenous insects into the laboratory beyond 4-7 d.

Anthopotamus verticis and *Stenonema spp*. mortality to Ni-spiked sediments was the most sensitive endpoint (Table 4-6). *Isonychia spp*. and *P. herricki* were less sensitive to Ni-spiked sediments than *A. verticis* and *Stenonema spp*. with both lethal and

sublethal endpoints (Table 4-6). Both *A. verticis* and *Stenonema spp*. were the two most sensitive indigenous organisms tested in the Ni-spiked sediment flow-thru tests. *Hyalella azteca* was the most sensitive species tested, and even with a longer duration test (10 d).

Liber et al. (2011) generated some LC_{50} and IC_{25} results for *H. azteca* and *C. dilutus* using Ni and a sediment type with much higher TOC (~7%) and higher AVS (1.7 μmol/g). Their *H. azteca* and *C. dilutus* results are summarized in Table 4-6. The sediment type Liber et al. (2011) used has ~3-10x more TOC and ~3x more AVS than GC sediments used in the flow-thru tests. The *H. azteca* and *C. dilutus* LC_{50} and IC_{25} Ni sensitivities in the flow-thru design were much lower than Liber et al. (2011) results (Table 4-6). This may be a function of TOC and AVS present in Liber et al. (2011) test sediments which should complex with Ni, and render it less bioavailable. These changes in Ni bioavailability can potentially cause these sediments to be less toxic than GC sediments in the flow-thru tests. However, there has been concern with static renewal Ni sediment toxicity tests, and the potential for organisms to be exposed to high Ni concentrations in the overlying water. Liber et al. (2011) stated that organisms are exposed to both porewater and overlying water metal concentrations in metal-sediment tests. They suggest that more frequent water changes are needed to flush this overlying water from the beaker systems (Liber et al. 2011). The flow-thru design was a novel alternative to static renewal tests, and may have provided more realistic exposures than those seen in static renewal designs.

Indigenous and surrogate organism responses compared to SEM/AVS models

Three of the four indigenous species Ni mortality responses fell outside the SEM_{Ni}/AVS model uncertainty range of 8-40 μmol/g, with the exception of *A. verticis*. (Table 4-8 and Fig 4-8). All of the indigenous species reference treatments were < 5.9 μmol/g for the SEM_{Ni}/AVS model (Table 4-1, Fig 4-8). Nearly all (except the highest Ni treatment for *A. verticis* and *Isonychia spp.*) of the indigenous species mortality responses fell within the bounds of uncertainty for the (SEM-AVS)/foc 150-3400 μmol/g (Fig 4-9). *Psephenus herricki* had the highest (SEM_{Ni}-AVS)/foc no effect value (> 3083) for all the indigenous species tested, and *A. verticis had* the lowest < 42.7 (Table 4-8, Fig 4-9). All of the indigenous species reference treatments were < 42.7 for (SEM_{Ni}-AVS)/foc (Table 4-8, Fig 4-9). *Hyalella azteca and C. dilutus* mortality had contrasting no effect SEM_{Ni}/AVS values, 18 and > 315, respectively (Table 4-8 and Fig 4-10). *Hyalella azteca and C. dilutus* mortality also had contrasting no effect (SEM_{Ni}-AVS)/foc values, 207 and > 1428, respectively (Table 4-8 and Fig 4-11).

The indigenous mortality effects in the Ni treatments all fell outside the range of uncertainty for SEM/AVS (8-40 μmol/g), and the reference treatments were all below 5.9. Using the 24% mortality effect level proposed by Berry et al. (1996) which the authors assessed 43 sediments and determined no effect levels were at < 24 % mortality when SEM/AVS < 1. Using this 24% mortality, there are clear effects being observed with *A. verticis* and *Stenonema spp.* in the Ni treatments (Fig 4-8). The results from the Dunnett's test, shows a large variation in no effect levels for the three SEM_{Ni}/AVS models. *Isonychia spp*. and *Psephenus herricki* were not as sensitive as *A. verticis* and

Stenonema spp. to Ni-spiked sediments in the flow-thru system. When looking at the SEM_{Ni}-AVS model, all the values were > 0 (no negative values), and this is represented by low AVS and TOC concentrations in GC sediments. The SEM_{Ni}-AVS model estimates of no effect levels were also varied, but *A. verticis* had lower no effect SEM_{Ni}-AVS value than *H. azteca*.

The $(SEM_{Ni}\text{-}AVS)/foc$ model mortality effects all fell within the range of uncertainty 150-3400 μmol/g (McGrath et al. 2002, USEPA 2005). The use of the SEM_{Ni}:AVS ratio has been suggested to be replaced by the SEM_{Ni}-AVS model (USEPA 2005), however, this model has still being used in current research (Costello et al. 2011, Paixao et al. 2010, Chapter 3). *Anthopotamus verticis* SEM_{Ni}/AVS model estimates were the lowest of all species tested. This would suggest that *A. verticis* was as sensitive to bioavailable Ni in the Ni-spiked sediment flow-thru tests as was *H. azteca*. The lack of negative values in the SEM_{Ni}-AVS and $(SEM_{Ni}\text{-}AVS)/foc$ models suggests that Ni is much more bioavailable than other sediment types which have higher AVS and TOC content (i.e. Warden Ditch or Big Beavercreek).

5-0 GENERAL CONCLUSIONS

The objectives of this study were to compare four indigenous aquatic insects with two USEPA surrogate organism responses (lethal and sublethal) to Ni-spiked sediments in flow-thru exposures. Also, an abbreviated method for simultaneously extracted metal (SEM_{Ni}) was compared to the standard USEPA SEM/AVS method. These objectives

were met by determining Ni-sediment threshold effect levels for the six organisms in the flow-thru system. Also, by demonstrating the abbreviated SEM_{Ni} extraction method was similar to SEM_{Ni} values obtained from the full AVS/SEM method. The SEM_{Ni} abbreviated extraction method appears to be a valid alternative to the full SEM/AVS method when analyzing sediments with low AVS content.

The hypotheses were not completely supported due to the Ni sensitivities of indigenous aquatic insect were varied in the Ni-sediment flow-thru exposures. *Isonychia spp*. was not the most sensitive to Ni based on LC_{10} and IC_{25} results. The mayflies *A. verticis* (burrower/filterer) and *Stenonema spp.* (grazer/scraper) were the most sensitive to Ni-spiked sediments. Overall, *H. azteca* was the most sensitive to Ni based on threshold effect levels (LC_{10} and IC_{25}) in the flow-thru tests. However, *A. verticis* SEM_{Ni}/AVS model responses were suggesting that this indigenous insect was as sensitive to Ni-spiked sediments as *H. azteca* in the flow-thru design. These results also suggest that survival and dry weights are sensitive endpoints for the indigenous species, and that dry weight instead of AFDW for *C. dilutus* was sufficient in these sediments which have low AVS and TOC. However, additional research is needed for testing this endpoint in these types of sediments.

Static-renewal testing has dominated sediment toxicity testing and data collection over the years. Recently, concerns with false positive results from increasing metal concentrations in the overlying water have been raised. This has challenged researchers to develop new tools for characterizing sediment toxicity without increased metal

concentrations in the overlying water. In a flow-thru design, these overlying water concentrations are minimized, and more realistic sediment exposure can be developed. This sediment toxicity data could contribute to sediment quality guidelines, and provide more precise sediment metal toxicity results.

Table 4-1. Indigenous organism sediment chemistry data from the 7 d Ni sediment flow-thru exposures.

Ni Treatment	Test organism	Date	SEM_{Ni}/AVS	(SEMNi-AVS)/f_{OC}	(SEM_{Ni}-AVS)	Total Ni	Total Ni	SEM_{Ni}	AVS	Total Mn	Total Fe	TOC	Hardness	Alkalinity
			(µmol/g)	(µmol/g)	(µmol/g)	(mg/kg)	(µmol/g)	(µmol/g)	(µmol/g)	(µmol/g)	(µmol/g)	(%)	(mg/L of $CaCO_3$)	(mg/L of $CaCO_3$)
GC-Ref	*Anthopotamus verticis*	11-Sep-08	6	43	0	22	0.37	0.23	0.04	6	96	0.4	**182**	**171**
GC-401	*Anthopotamus verticis*	11-Sep-08	129	654	7	401	6.83	6.66	0.05	7	101	1.0		
GC-769	*Anthopotamus verticis*	11-Sep-08	302	602	14	769	13.10	14.54	0.05	7	108	2.4		
GC-1254	*Anthopotamus verticis*	11-Sep-08	316	1095	21	1254	21.36	20.76	0.07	9	116	1.9		
GC-3276	*Anthopotamus verticis*	11-Sep-08	754	1787	46	3276	55.81	46.27	0.06	6	126	2.6		
GC-6104	*Anthopotamus verticis*	11-Sep-08	1425	3505	80	6104	103.99	80.18	0.06	7	161	2.3		
GC-Ref	*Anthopotamus verticis*	18-Sep-08	8	45	0	19	0.32	0.20	0.03	7	108	0.4	**182**	**171**
GC-401	*Anthopotamus verticis*	18-Sep-08	123	1064	5	312	5.31	4.59	0.04	8	123	0.4		
GC-769	*Anthopotamus verticis*	18-Sep-08	146	1586	9	538	9.17	9.08	0.06	8	98	0.6		
GC-1254	*Anthopotamus verticis*	18-Sep-08	429	1240	12	779	13.27	11.81	0.03	7	86	1.0		
GC-3276	*Anthopotamus verticis*	18-Sep-08	232	942	10	958	16.33	9.85	0.04	7	105	1.0		
GC-6104	*Anthopotamus verticis*	18-Sep-08	699	1913	37	1442	24.57	37.35	0.05	6	88	1.9		
GC-Ref	*Psphenus herricki*	25-Sep-08	6	26	0	18	0.31	0.18	0.03	7	135	0.6	**180**	**158**
GC-436	*Psphenus herricki*	25-Sep-08	147	753	6	436	7.44	6.53	0.04	8	136	0.9		
GC-777	*Psphenus herricki*	25-Sep-08	221	626	12	777	13.24	12.14	0.06	8	92	1.9		
GC-1460	*Psphenus herricki*	25-Sep-08	295	591	14	1460	24.86	13.78	0.05	6	102	2.3		
GC-3287	*Psphenus herricki*	25-Sep-08	928	1531	48	3287	56.00	48.19	0.05	8	127	3.1		
GC-6616	*Psphenus herricki*	25-Sep-08	1747	3083	96	6616	112.70	95.80	0.05	6	105	3.1		
GC-Ref	*Stenonema spp.*	9-Oct-08	6	22	0	16	0.27	0.18	0.03	6	151	0.7	**193**	**165**
GC-539	*Stenonema spp.*	9-Oct-08	124	434	5	539	9.18	5.53	0.04	6	153	1.3		
GC-790	*Stenonema spp.*	9-Oct-08	268	715	15	790	13.45	14.73	0.06	6	123	2.1		
GC-2107	*Stenonema spp.*	9-Oct-08	559	981	26	2107	35.90	26.04	0.05	6	158	2.7		
GC-3257	*Stenonema spp.*	9-Oct-08	1032	1821	54	3257	55.49	53.63	0.05	6	111	2.9		
GC-6712	*Stenonema spp.*	9-Oct-08	1942	3122	106	6712	114.35	106.50	0.05	6	110	3.4		
GC-Ref	*Isonychia spp.*	23-Oct-08	4	14	0	18	0.31	0.13	0.03	5	104	0.7	**192**	**170**
GC-482	*Isonychia spp.*	23-Oct-08	170	941	8	482	8.21	7.55	0.04	7	128	0.8		
GC-799	*Isonychia spp.*	23-Oct-08	227	823	12	799	13.61	12.48	0.06	6	95	1.5		
GC-1166	*Isonychia spp.*	23-Oct-08	567	1135	26	1166	19.87	26.42	0.05	7	107	2.3		
GC-2354	*Isonychia spp.*	23-Oct-08	792	1335	41	2354	40.11	41.16	0.05	7	139	3.1		
GC-6056	*Isonychia spp.*	23-Oct-08	1721	3433	94	6056	103.16	94.37	0.05	6	121	2.7		

AVS values for *P. herricki*, *Stenonema spp.*, and *Isonychia spp.* tests was the mean of Day 0 and Day 7 from the *A. verticis* test

Table 4-2. Surrogate organism sediment chemistry data from the 10 d Ni sediment flow-thru exposures.

Ni Treatment	Test organism	Date	SEM_{Ni}/AVS	(SEMNi-AVS)/*foc*	(SEM_{Ni}-AVS)	Total Ni	Total Ni	SEM_{Ni}	AVS	Total Mn	Total Fe	TOC	Hardness	Alkalinity
			(µmol/g)	(µmol/g)	(µmol/g)	(mg/kg)	(µmol/g)	(µmol/g)	(µmol/g)	(µmol/g)	(µmol/g)	(%)	(mg/L of $CaCO_3$)	(mg/L of $CaCO_3$)
GC-Ref	*Chironomus dilutus*	5-Dec-08	5	80	0	13	0.22	0.16	0.03	5	89	0.2	**197**	**191**
GC-76	*Chironomus dilutus*	5-Dec-08	26	1954	1	76	1.29	1.15	0.04	6	101	0.1		
GC-109	*Chironomus dilutus*	5-Dec-08	30	303	2	109	1.86	1.66	0.06	5	108	0.5		
GC-300	*Chironomus dilutus*	5-Dec-08	72	2366	3	300	5.11	3.35	0.05	9	522	0.1		
GC-462	*Chironomus dilutus*	5-Dec-08	149	868	8	462	7.88	7.73	0.05	6	92	0.9		
GC-922	*Chironomus dilutus*	5-Dec-08	315	1428	17	922	15.70	17.25	0.05	5	107	1.2		
GC-Ref	*Chironomus dilutus*	15-Dec-08	2	169	0	16	0.27	0.15	0.03	7	132	0.1		
GC-76	*Chironomus dilutus*	15-Dec-08	31	658	1	43	0.73	0.84	0.04	5	89	0.1		
GC-109	*Chironomus dilutus*	15-Dec-08	146	518	1	93	1.58	1.37	0.06	5	95	0.3		
GC-300	*Chironomus dilutus*	15-Dec-08	439	388	2	95	1.61	1.81	0.05	6	130	0.5		
GC-462	*Chironomus dilutus*	15-Dec-08	202	956	4	355	6.05	4.22	0.05	5	105	0.4		
GC-922	*Chironomus dilutus*	15-Dec-08	693	2304	10	597	10.18	10.09	0.05	7	121	0.4		
GC-Ref	*Hyalella azteca*	6-Jan-09	5	30	0	13	0.22	0.15	0.03	5	114	0.4	**197**	**191**
GC-26	*Hyalella azteca*	6-Jan-09	14	148	1	26	0.45	0.62	0.04	7	92	0.4		
GC-43	*Hyalella azteca*	6-Jan-09	18	207	1	43	0.73	0.99	0.06	5	126	0.5		
GC-125	*Hyalella azteca*	6-Jan-09	40	492	2	125	2.13	1.87	0.05	7	132	0.4		
GC-197	*Hyalella azteca*	6-Jan-09	80	556	4	197	3.36	4.13	0.05	4	86	0.7		
GC-676	*Hyalella azteca*	6-Jan-09	143	870	8	676	11.51	7.82	0.05	7	117	0.9		
GC-Ref	*Hyalella azteca*	16-Jan-09	4	22	0	16	0.27	0.13	0.03	8	105	0.4		
GC-26	*Hyalella azteca*	16-Jan-09	11	91	0	26	0.44	0.47	0.04	6	98	0.5		
GC-43	*Hyalella azteca*	16-Jan-09	15	151	1	59	1.01	0.80	0.06	5	98	0.5		
GC-125	*Hyalella azteca*	16-Jan-09	40	536	2	109	1.86	1.87	0.05	6	95	0.3		
GC-197	*Hyalella azteca*	16-Jan-09	65	1105	3	198	3.38	3.36	0.05	7	194	0.3		
GC-676	*Hyalella azteca*	16-Jan-09	86	1232	5	261	4.44	4.70	0.05	6	105	0.4		

AVS values was the mean of Day 0 and Day 7 from the *A. verticis* test

Table 4-3. Percent change for three Ni sediment tests, and Ni loss is seen after 7 (*Anthopotamus verticis* test) and 10 d (*Hyalella azteca* and *Chironomus dilutus* tests).

Ni Treatment	Test organism	Date	Total Ni	SEM_{Ni}	AVS	Total Mn	Total Fe	TOC
			(µmol/g)	(µmol/g)	(µmol/g)	(mg/kg)	(mg/kg)	(%)
GC-Ref	Anthopotamus verticis	11-Sep-08	-14	-12	-34	9	13	-13
GC-401	Anthopotamus verticis	11-Sep-08	-22	-31	-27	13	21	-58
GC-769	Anthopotamus verticis	11-Sep-08	-30	-38	29	22	-9	-76
GC-1254	Anthopotamus verticis	11-Sep-08	-38	-43	-58	-21	-26	-50
GC-3276	Anthopotamus verticis	11-Sep-08	-71	-79	-31	13	-17	-60
GC-6104	Anthopotamus verticis	11-Sep-08	-76	-53	-5	-9	-45	-15
GC-Ref	Chironomus dilutus	5-Dec-08	23	-4	*	52	48	-55
GC-76	Chironomus dilutus	5-Dec-08	-44	-27	*	-11	-12	114
GC-109	Chironomus dilutus	5-Dec-08	-15	-17	*	7	-12	-52
GC-300	Chironomus dilutus	5-Dec-08	-68	-46	*	-39	-75	225
GC-462	Chironomus dilutus	5-Dec-08	-23	-45	*	-13	14	-51
GC-922	Chironomus dilutus	5-Dec-08	-35	-42	*	28	12	-64
GC-Ref	Hyalella azteca	6-Jan-09	23	-15	*	50	-7	11
GC-26	Hyalella azteca	6-Jan-09	0	-24	*	-20	6	20
GC-43	Hyalella azteca	6-Jan-09	38	-19	*	2	-22	10
GC-125	Hyalella azteca	6-Jan-09	-13	0	*	-8	-28	-8
GC-197	Hyalella azteca	6-Jan-09	0	-19	*	51	126	-59
GC-676	Hyalella azteca	6-Jan-09	-61	-40	*	-6	-10	-58

*AVS values was the mean of Day 0 and Day 7 from the *A. verticis* test

Table 4-4. Physico-chemical data from all Ni sediment flow-thru tests. Ni treatment (far left column) is designated at Greenville Creek (GC) and Ni concentration in mg/kg, e.g. GC-401. All data is presented as mean and standard deviation (St. Dev).

Ni Treatment	Test organism	Date	Temperature (Mean)	Temperature (St. Dev)	DO (Mean)	DO (St. Dev)	Conductivity (Mean)	Conductivity (St. Dev)	pH (Mean)	pH (St. Dev)	Sediment pH (Mean)	Sediment pH (St. Dev)	Sediment Temp (Mean)	Sediment Temp (St. Dev)	Hardness	Alkalinity	DOC
			°C	°C	(mg/L)	(mg/L)	(µS/cm)	(µS/cm)	units	units	units	units	°C	°C	(mg/L of $CaCO_3$)	(mg/L of $CaCO_3$)	mg/L
GC-Ref	*Anthopotamus verticis*	11-Sep-08	22.2	0.4	7.63	0.33	389	74	7.83	0.07	7.60	0.05	21.4	0.2	182	171	0.7
GC-401	*Anthopotamus verticis*	11-Sep-08	22.0	0.3	7.65	0.32	387	69	7.87	0.06	6.56	0.10	21.3	0.1			1.2
GC-769	*Anthopotamus verticis*	11-Sep-08	22.0	0.4	7.62	0.31	388	71	7.85	0.10	6.12	0.04	21.5	0.3			0.9
GC-1254	*Anthopotamus verticis*	11-Sep-08	22.1	0.4	7.62	0.32	393	73	7.84	0.07	5.73	0.08	21.5	0.2			1.0
GC-3276	*Anthopotamus verticis*	11-Sep-08	22.1	0.4	7.63	0.34	394	72	7.84	0.06	5.59	0.05	21.5	0.2			0.8
GC-6104	*Anthopotamus verticis*	11-Sep-08	22.0	0.4	7.65	0.32	396	70	7.86	0.06	5.09	0.51	21.4	0.2			0.8
GC-Ref	*Psphenus herricki*	25-Sep-08	21.8	0.7	7.39	0.55	405	24	7.83	0.21	7.57	0.15	20.8	0.6	180	158	1.8
GC-436	*Psphenus herricki*	25-Sep-08	22.1	0.5	7.32	0.58	407	23	7.87	0.21	6.77	0.10	20.8	0.5			1.7
GC-777	*Psphenus herricki*	25-Sep-08	22.2	0.5	7.28	0.58	405	24	7.87	0.21	6.43	0.04	20.7	0.5			1.9
GC-1460	*Psphenus herricki*	25-Sep-08	22.3	0.3	7.07	0.56	409	28	7.94	0.30	6.09	0.08	20.9	0.3			1.7
GC-3287	*Psphenus herricki*	25-Sep-08	22.2	0.5	7.36	0.56	407	26	7.90	0.21	5.68	0.06	20.7	0.4			2.4
GC-6616	*Psphenus herricki*	25-Sep-08	22.2	0.5	7.31	0.59	413	24	7.89	0.21	5.38	0.02	20.7	0.4			1.6
GC-Ref	*Stenonema spp.*	9-Oct-08	21.6	0.3	7.55	0.32	428	35	7.78	0.15	7.59	0.06	20.8	0.4	193	165	2.2
GC-539	*Stenonema spp.*	9-Oct-08	21.8	0.4	7.50	0.35	426	39	7.79	0.15	6.52	0.38	20.9	0.6			1.8
GC-790	*Stenonema spp.*	9-Oct-08	21.8	0.5	7.41	0.45	425	37	7.85	0.27	5.95	0.01	20.8	0.6			1.5
GC-2107	*Stenonema spp.*	9-Oct-08	21.8	0.4	7.37	0.50	426	37	7.87	0.28	5.72	0.09	20.7	0.6			1.5
GC-3257	*Stenonema spp.*	9-Oct-08	21.9	0.5	7.36	0.47	436	53	7.88	0.22	5.58	0.12	20.8	0.6			1.5
GC-6712	*Stenonema spp.*	9-Oct-08	21.9	0.5	7.34	0.42	438	50	7.88	0.23	5.32	0.10	20.8	0.7			2.1
GC-Ref	*Isonychia spp.*	23-Oct-08	20.3	0.5	8.46	0.26	437	42	7.79	0.08	7.61	0.19	19.5	0.2	192	170	2.1
GC-482	*Isonychia spp.*	23-Oct-08	20.4	0.4	8.42	0.21	435	41	7.80	0.08	6.75	0.17	19.5	0.2			1.4
GC-799	*Isonychia spp.*	23-Oct-08	20.4	0.2	8.45	0.24	436	40	7.84	0.09	6.11	0.07	19.4	0.2			2.4
GC-1166	*Isonychia spp.*	23-Oct-08	20.4	0.2	8.40	0.22	437	40	7.83	0.08	5.90	0.05	19.4	0.2			2.7
GC-2354	*Isonychia spp.*	23-Oct-08	20.5	0.3	8.39	0.18	439	41	7.87	0.09	5.58	0.09	19.5	0.2			1.4
GC-6056	*Isonychia spp.*	23-Oct-08	20.5	0.3	8.36	0.20	442	41	7.86	0.08	5.31	0.15	19.5	0.2			1.6
GC-Ref	*Chironomus dilutus*	5-Dec-08	20.8	0.9	8.19	0.38	374	62	7.82	0.10	7.71	0.23	19.7	0.7	197	191	2.6
GC-76	*Chironomus dilutus*	5-Dec-08	21.0	0.8	8.19	0.42	372	61	7.84	0.10	7.44	0.09	19.7	0.6			2.4
GC-109	*Chironomus dilutus*	5-Dec-08	21.0	0.8	8.10	0.43	372	63	7.86	0.10	7.25	0.03	19.7	0.7			2.2
GC-300	*Chironomus dilutus*	5-Dec-08	21.0	0.8	8.03	0.47	371	63	7.87	0.09	7.13	0.13	19.6	0.7			2.6
GC-462	*Chironomus dilutus*	5-Dec-08	20.9	0.8	8.14	0.45	373	63	7.89	0.09	6.34	0.09	19.6	0.8			2.6
GC-922	*Chironomus dilutus*	5-Dec-08	20.9	0.9	8.18	0.41	375	65	7.89	0.08	6.05	0.14	19.4	0.9			2.0
GC-Ref	*Hyalella azteca*	6-Jan-09	21.4	0.9	8.09	0.31	370	37	7.76	0.10	7.65	0.17	20.2	0.71	189	185	2.9
GC-26	*Hyalella azteca*	6-Jan-09	21.4	0.9	8.12	0.33	367	38	7.77	0.10	7.29	0.20	20.2	0.76			1.7
GC-43	*Hyalella azteca*	6-Jan-09	21.4	0.8	8.08	0.35	368	38	7.77	0.09	7.23	0.24	20.1	0.96			2.1
GC-125	*Hyalella azteca*	6-Jan-09	21.4	0.7	8.11	0.33	367	37	7.79	0.09	6.77	0.22	20.1	0.78			2.2
GC-197	*Hyalella azteca*	6-Jan-09	21.4	0.8	8.10	0.37	366	37	7.78	0.07	6.61	0.39	20.2	0.75			2.2
GC-676	*Hyalella azteca*	6-Jan-09	21.3	0.8	8.03	0.36	366	37	7.80	0.08	6.14	0.40	20.1	0.80			2.2

Table 4-5. Statistical results from the three SEM_{Ni} extraction comparison tests. The comparison was between the full AVS method versus a shaker method which determines SEM_{Ni}. The GC sediments are very low in AVS while BC and WD sediments have moderately high and very high AVS content, respectively. No statistical differences were observed for SEM_{Ni}, and the AVS concentrations in GC sediments were then assumed to be similar for use SEM_{Ni}/AVS model estimates throughout the remaining tests.

Treatment	SEM-Ni mean (µmol/g)	SEM-Ni St.dev (µmol/g)	*p* -value
GC Sediment AVS Method	6.3	0.1	0.328
GC Sediment Shaker Method	6.4	0.4	
BC Sediment AVS Method	22.9	0.7	0.478
BC Sediment Shaker Method	22.9	0.2	
WD Sediment AVS Method	51.9	1.8	0.07
WD Sediment Shaker Method	49.3	1.3	

GC = Greenville Creek

BC = Big Beavercreek

WD = Warden Ditch

Table 4-6. Threshold effect concentrations for a host of endpoints for all organisms used in the Ni sediment flow-thru toxicity tests. Survival and growth endpoints were calculated using EC_{10} and IC_{25} threshold effect levels, respectively.

	Endpoint			
	Survival (LC_{10}) µmol/g Ni	Length (IC_{25}) µmol/g Ni	Head Capsule Width (IC_{25}) µmol/g Ni	Survival (LC_{50}) µmol/g Ni
A. verticis Day 0	3.2 (1.4, 5.1)	59.0 (17.6, 73.2)*	59.4 (17.4, 72.9)*	
A. verticis Day 7	3.7 (0.4, 6.2)	16.9 (12.8, 19.3)*	16.9 (12.7, 19.3)*	
P. herricki Day 0	nr	> 112.7 *	**	
Stenonema spp. Day 0	6.0 (3.2, 8.8)	42.2 (39.1, 57.8)*	42.0 (38.3, 53.7)*	
Isonychia spp. Day 0	19.8 (7.1, 35.6)	> 103.2	> 103.2	
C. dilutus Day 0	>15.7	**	**	>15.7
C. dilutus Day 10	>10.2	**	**	>10.2
H. azteca Day 0	0.4 (0, 1.0)	**	**	2.2 (0.7, 6.4)
H. azteca Day 10	0.5 (0.3, 0.8)	**	**	1.9 (1.5, 2.3)
C. dilutus †				>56
H. azteca †				8.9 (8.3, 9.5)
	Dry weight (IC_{25}) µmol/g Ni	AFDW (IC_{25}) µmol/g Ni	Exuvia (IC_{25}) µmol/g Ni	
A. verticis Day 0	6.3 (3.2, 21.9)	13.1 (0, 23.6)	**	
A. verticis Day 7	4.9 (3.0, 14.2)	9.2 (0.4, 14.1)	**	
P. herricki Day 0	> 112.7 *	> 112.7 *	**	
Stenonema spp. Day 0	10.5 (3.3, 21.3)	11.0 (4.0, 20.2)	14.6 (0.4, 30.7)	
Isonychia spp. Day 0	> 103.2	> 103.2	19.0 (nr)	
C. dilutus Day 0	2.2 (1.0, 4.9)	4.7 (0, 7.2)	**	
C. dilutus Day 10	1.6 (0.9, 1.6)	1.6 (1.0, 4.7)	**	
H. azteca Day 0	0.6 (0.3, 1.8)	**	**	
H. azteca Day 10	0.8 (0.3, 1.8)	**	**	
C. dilutus †	9.2 (7.0, 12.1)			
H. azteca †	3.2 (2.7, 4.1)			

nr (no result)
*assumptions violated
** not measured
† results from Liber et al. 2011

Table 4-7. Growth data for all organisms used in the Ni sediment flow-thru toxicity tests. Treatment (GC-xxxx) results are listed for lengths, head capsules widths, dry and Ash-free dry weights (AFDW), and exuvia for selected organisms. One-way ANOVA results indicate significant treatment effects from Tukey's pairwise comparisons (α = 0.05).

Species	Endpoint	Ni Treatment (mg/kg)						
		GC-Ref	**GC-401**	**GC-769**	**GC-1254**	**GC-3276**	**GC-6104**	**ANOVA**
Anthopotamus verticis	% Survival	98	65	58	40	18	0	$p < 0.001$
	St.dev	5	17	10	26	13	0	
	Length (mm)	6.84	6.85	7.19	6.88	7.44	0.00	$p = 0.01$
	St.dev	0.21	0.11	0.17	0.69	0.07	0.00	
	Head width (mm)	1.32	1.40	1.41	1.41	1.13	0.00	
	St.dev	0.02	0.02	0.06	0.15	0.00	0.00	
	Dry wt (mg)	10.28	7.27	7.65	4.98	2.58	0.00	$p < 0.001$
	St.dev	1.63	1.76	1.34	2.67	1.94	0.00	
	AFDW (mg)	8.98	6.42	7.05	4.58	2.37	0.00	$p < 0.001$
	St.dev	1.37	1.65	1.45	2.54	1.76	0.00	
		GC-Ref	**GC-436**	**GC-777**	**GC-1460**	**GC-3287**	**GC-6616**	
Psphenus herricki	% Survival	88	93	95	93	90	88	
	St.dev	5	10	6	6	14	10	
	Length (mm)	8.11	7.65	7.87	7.78	7.84	7.84	
	St.dev	0.28	0.29	0.13	0.38	0.11	0.24	
	Dry wt (mg)	56.55	56.80	67.23	62.07	63.55	62.00	
	St.dev	10.22	11.85	8.65	8.51	13.93	4.59	
	AFDW (mg)	54.28	54.43	64.43	59.50	60.87	58.20	
	St.dev	9.92	11.45	8.25	8.05	13.62	3.70	
		GC-Ref	**GC-539**	**GC-790**	**GC-2107**	**GC-3257**	**GC-6712**	
Stenonema spp.	% Survival	93	75	53	40	10	0	$p < 0.001$
	St.dev	10	13	15	26	20	0	
	Length (mm)	3.92	4.06	4.04	4.33	3.75	0.00	
	St.dev	0.11	0.33	0.58	0.53	0.50	0.00	
	Head width (mm)	1.39	1.45	1.52	1.44	1.25	0.00	$p = 0.008$
	St.dev	0.08	0.07	0.22	0.21	0.00	0.00	
	Dry wt (mg)	6.17	5.00	3.77	2.28	0.73	0.00	$p < 0.001$
	St.dev	0.51	1.39	1.48	1.16	1.45	0.00	
	AFDW (mg)	5.53	4.65	3.45	1.87	2.60	0.00	$p = 0.002$
	St.dev	0.38	1.38	1.34	1.04	0.00	0.00	
	Exuvia	6.5	5.3	5.0	2.5	1.0	1.0	$p < 0.001$
	St.dev	2.1	1.5	1.2	1.0	1.4	0.0	
		GC-Ref	**GC-482**	**GC-799**	**GC-1166**	**GC-2354**	**GC-6056**	
Isonychia spp.	% Survival	100	95	95	90	80	78	$p = 0.026$
	St.dev	0	6	10	10	8	17	
	Length (mm)	7.40	6.99	7.35	7.18	7.17	7.08	
	St.dev	0.24	0.41	0.26	0.29	0.24	0.44	
	Head width (mm)	1.05	1.03	1.00	1.03	1.03	1.01	
	St.dev	0.12	0.09	0.00	0.00	0.10	0.08	
	Dry wt (mg)	15.98	13.68	15.60	13.28	13.60	12.35	
	St.dev	0.62	2.58	3.00	4.12	3.34	2.84	
	AFDW (mg)	15.15	12.95	14.80	12.78	12.88	12.00	
	St.dev	0.69	2.40	2.74	4.04	3.12	2.61	
	Exuvia	8.0	7.0	8.0	5.8	5.3	5.3	
	St.dev	0.0	1.4	3.6	1.0	1.9	1.9	
		GC-Ref	**GC-76**	**GC-109**	**GC-300**	**GC-462**	**GC-922**	
Chironomus dilutus	% Survival	90	98	88	90	88	88	
	St.dev	14	5	25	10	13	10	
	Dry wt (mg)	11.25	11.53	8.75	6.55	4.82	3.20	$p < 0.001$
	St.dev	1.36	3.80	1.84	2.01	0.68	0.62	
	AFDW (mg)	6.62	7.48	5.72	5.23	3.53	2.43	$p = 0.005$
	St.dev	0.80	3.44	1.72	1.13	0.36	0.45	
		GC-Ref	**GC-26**	**GC-43**	**GC-125**	**GC-197**	**GC-676**	
Hyalella azteca	% Survival	95	98	65	50	30	20	$p < 0.001$
	St.dev	6	5	17	0	18	22	
	Dry wt (mg)	0.85	0.80	0.95	0.95	0.92	1.13	
	St.dev	0.21	0.26	0.24	0.24	0.13	0.15	

Endpoints are treatment means

GC = Greenville Creek

GC-Ni conc (mg/kg)

Table 4-8. The no effect levels of the three SEM_{Ni}/AVS models in the flow-thru tests. Dunnett's test results on survival data from all species used in the Ni sediment tests. Values are representing no toxicity below these levels. All values are based on Day 0 samples, and these may be over protective due to Ni flux being observed from sediments over the duration of the test.

Survival	SEM_{Ni}/AVS (µmol/g)	(SEM_{Ni}-AVS)/*foc* (µmol/g)	(SEM_{Ni}-AVS) (µmol/g)
Anthopotamus verticis (Day 0)	5.9	42.7	0.2
Psephenus herricki (Day 0)	>1747	>3083	>96
Stenonema spp. (Day 0)	125	434	5.5
Isonychia spp. (Day 0)	567	1135	26
Chironomus dilutus (Day 0)	>315	>2366	>17
Hyalella azteca (Day 0)	18	207	0.6

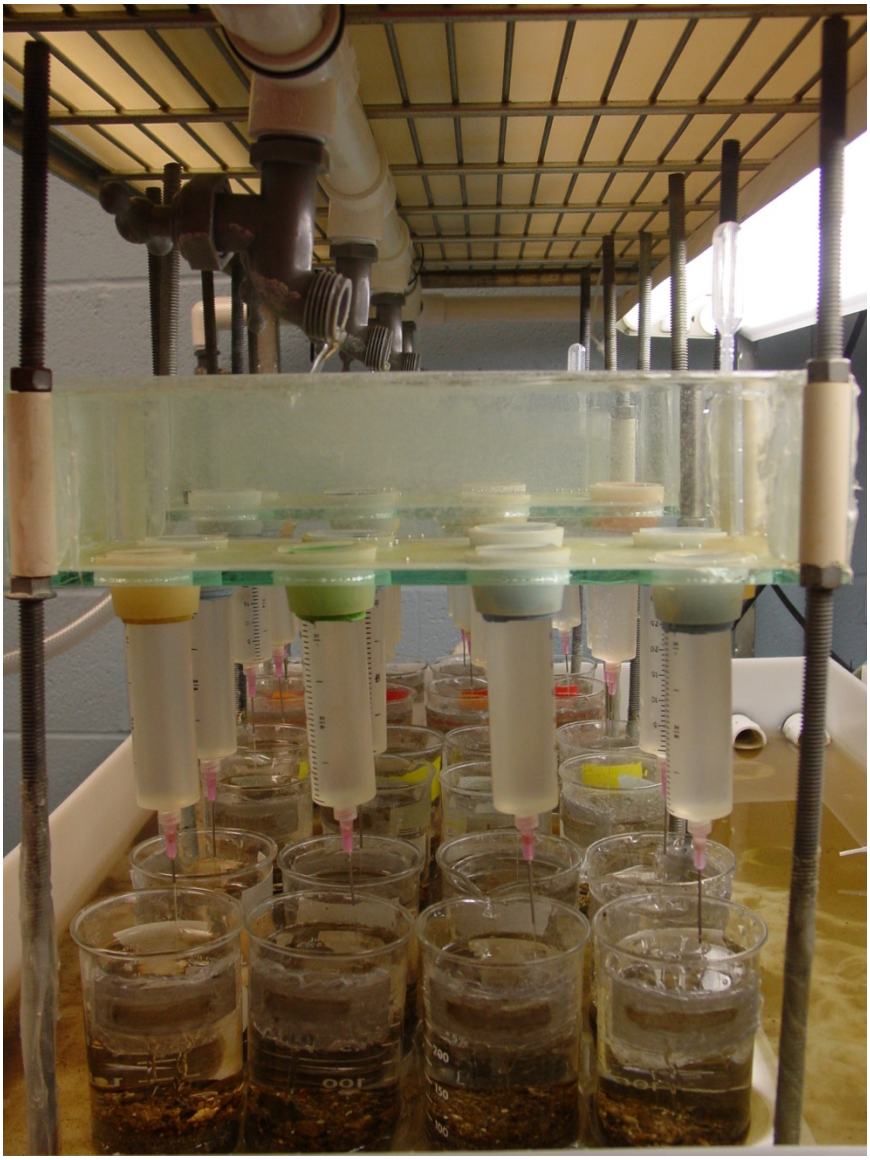

Figure 4-1. Ni flow-thru design: sediments receiving water from Zumwalt apparatus, and flowing out of beakers.

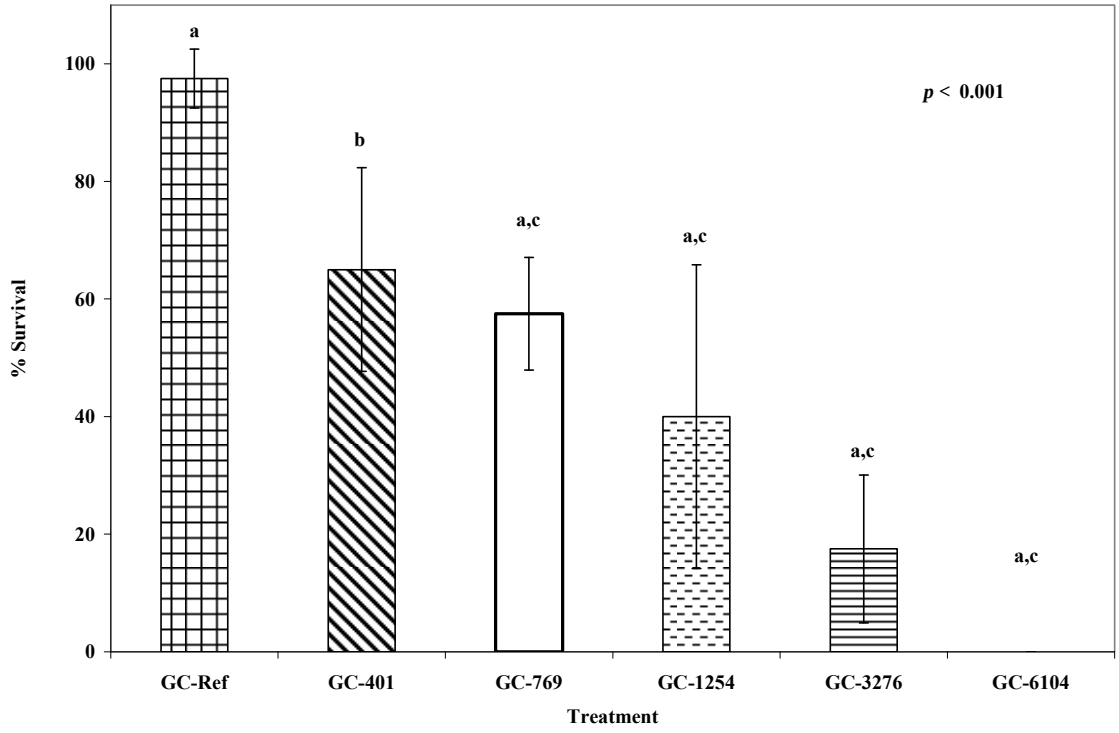

Figure 4-2. *Anthopotamus verticis* survival during the 7 d Ni sediment flow-thru toxicity test (11-Sept-08).

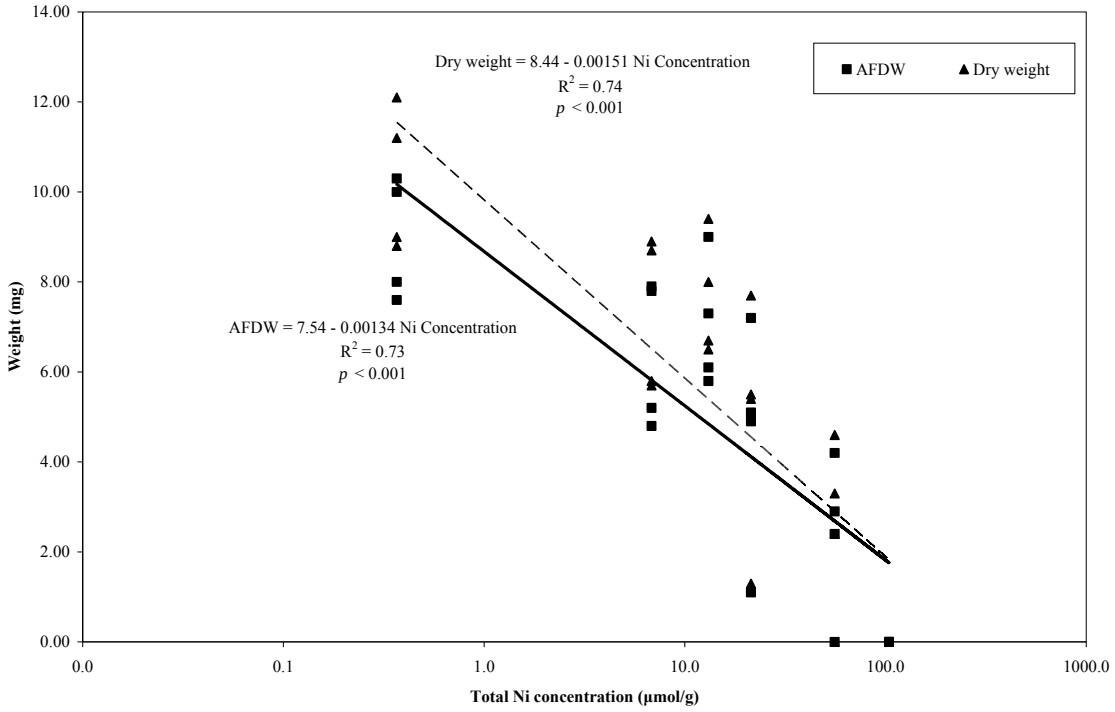

Figure 4-3. *Anthopotamus verticis* growth (AFDW and Dry Weight) responses to increasing log Ni concentrations during the 2008 Ni sediment Flow-thru exposures. Dark regression line is AFDW, and dashed regression line is Dry Weight.

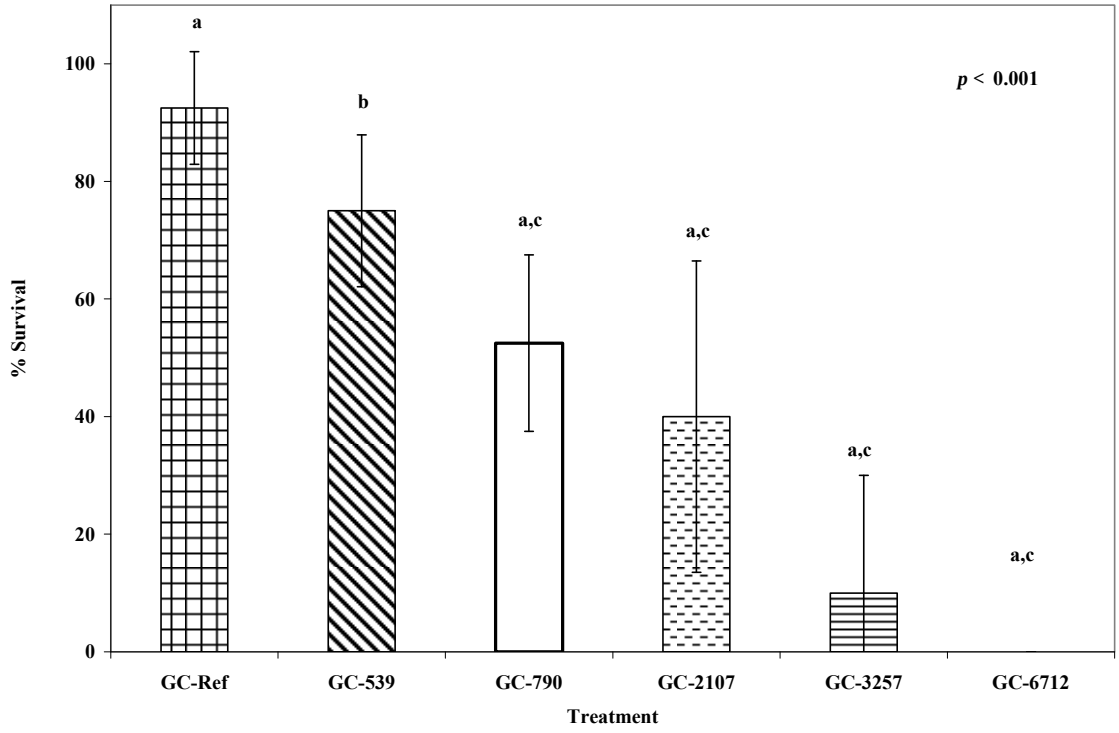

Figure 4-4. *Stenonema spp*. survival during the 7 d Ni sediment flow-thru toxicity test (9-Oct-08).

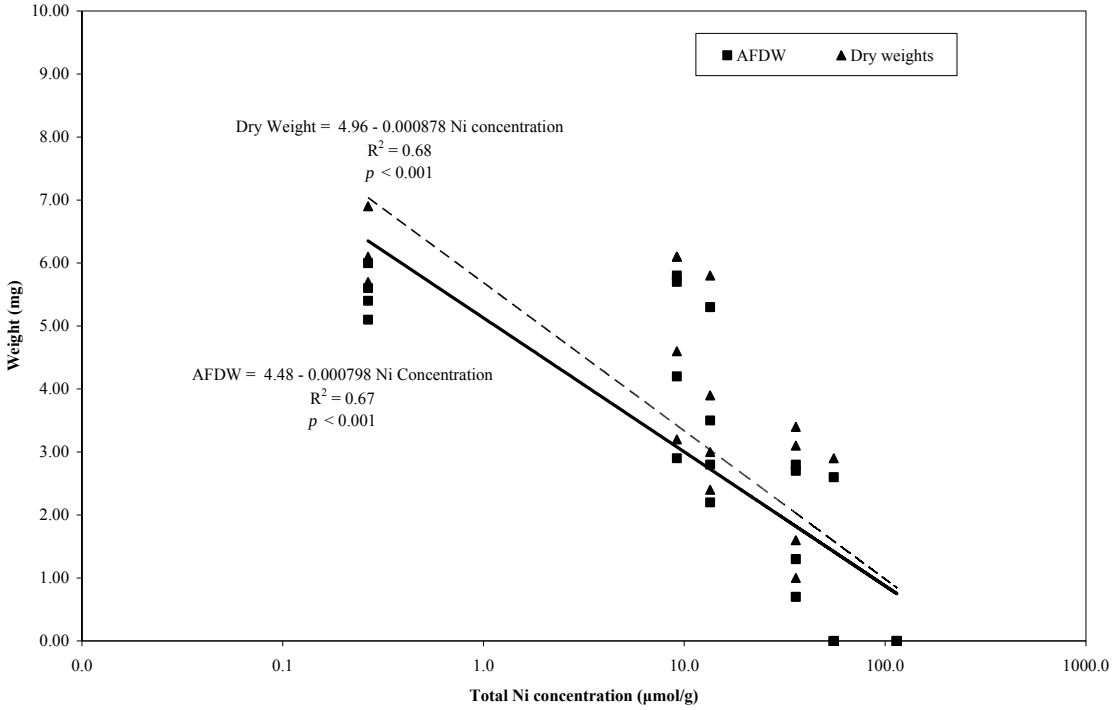

Figure 4-5. ***Stenonema spp.*** **growth (AFDW and Dry Weight) responses to increasing log Ni concentrations during the 2008 Ni sediment Flow-thru exposures. Dark regression line is AFDW, and dashed regression line is Dry Weight.**

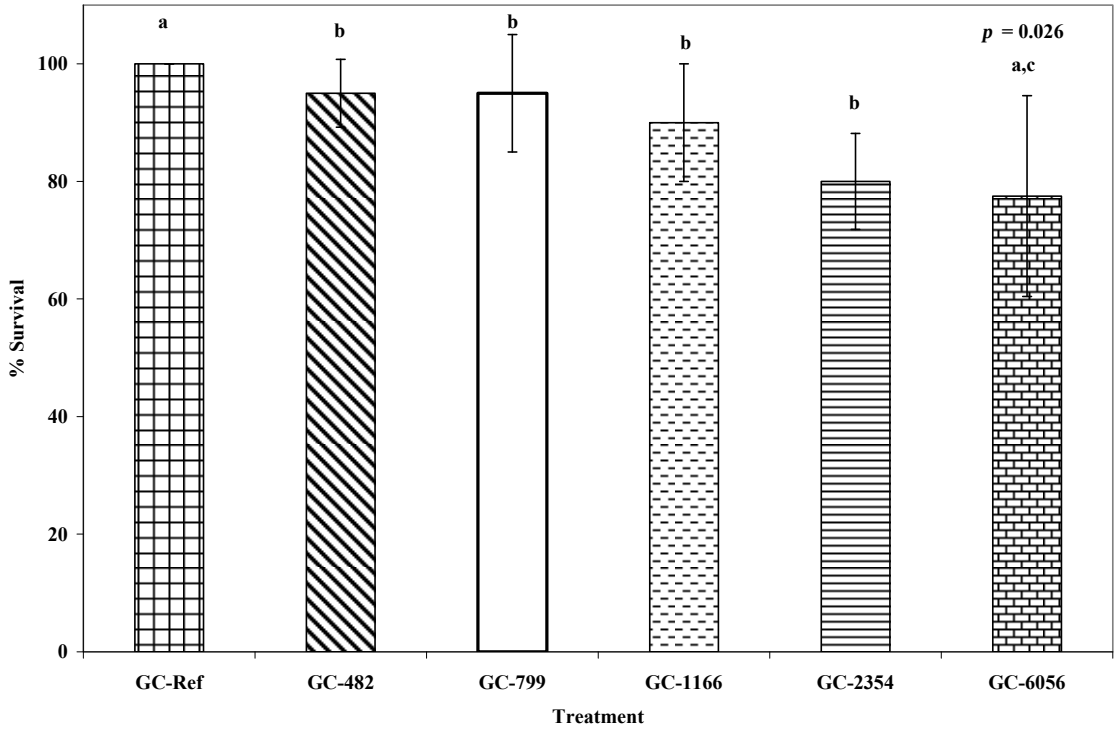

Figure 4-6. *Isonychia spp.* survival during the 7 d Ni sediment flow-thru toxicity test (23-Oct-08).

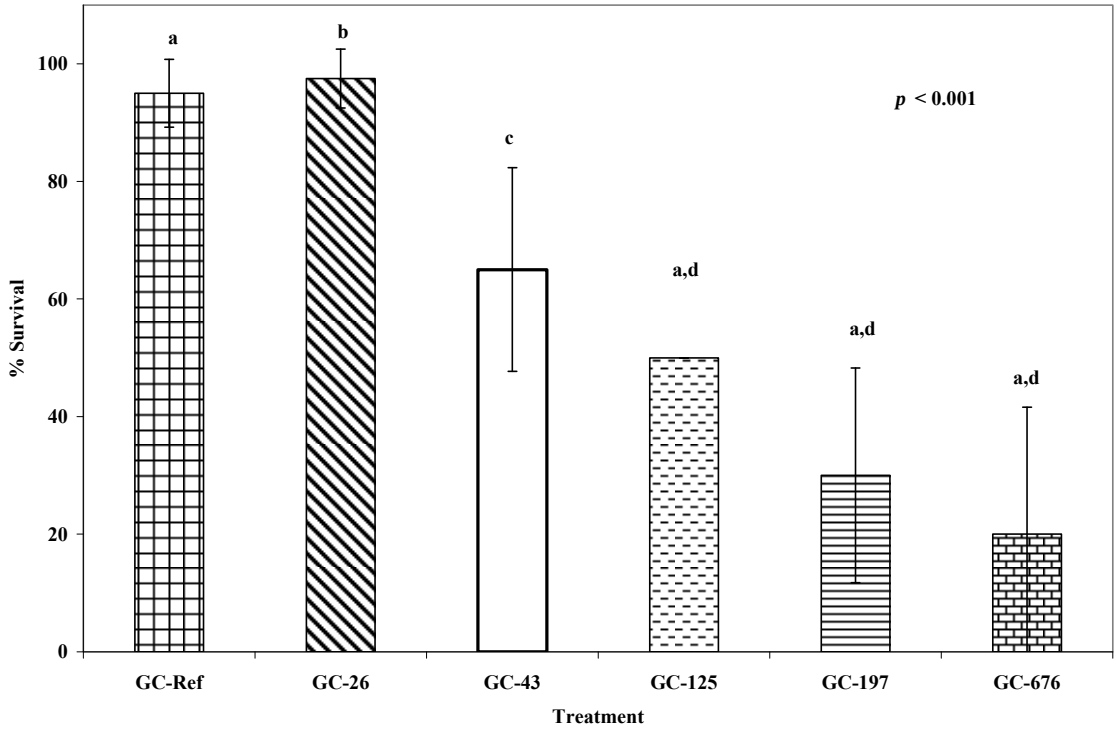

Figure 4-7. *Hyalella azteca* survival during the 10 d Ni sediment flow-thru toxicity test (6-Jan-09).

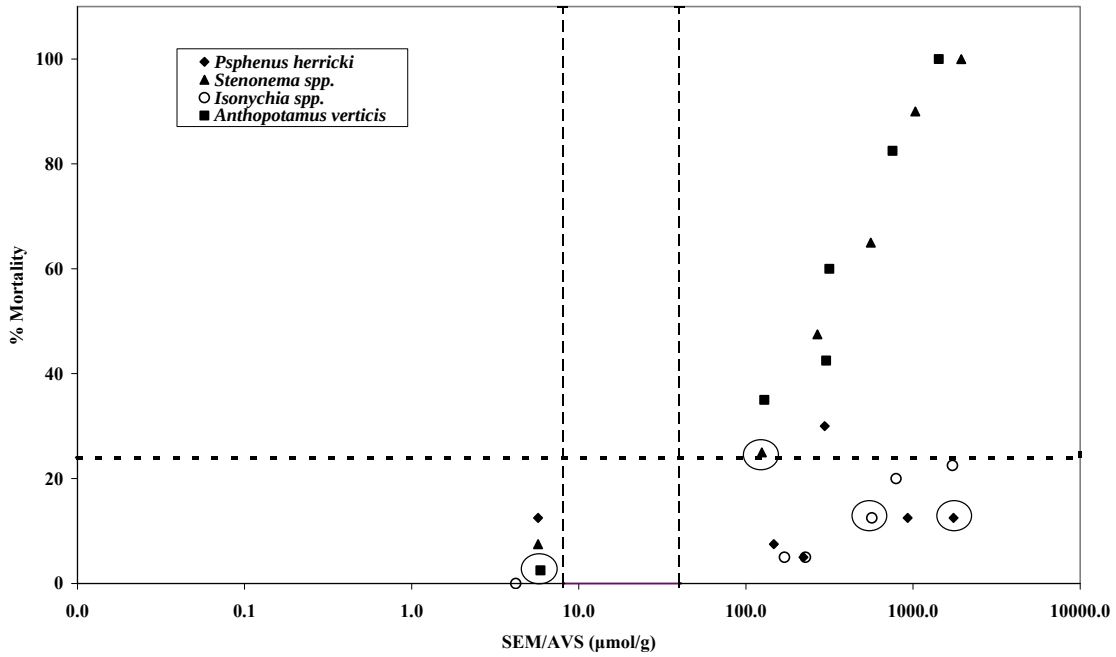

Figure 4-8. Indigenous organism mortality to log SEM_{Ni}/AVS values in the Ni sediment flow-thru exposures. Vertical dashed lines represent SEM_{Ni}/AVS 8 and 40, range of uncertainty, and horizontal dashed line is the 24% mortality level (Berry et al. 1996). All of the reference treatments were below SEM_{Ni}/AVS < 5.9. The circles represent the Dunnett's test results for the no effect treatment levels.

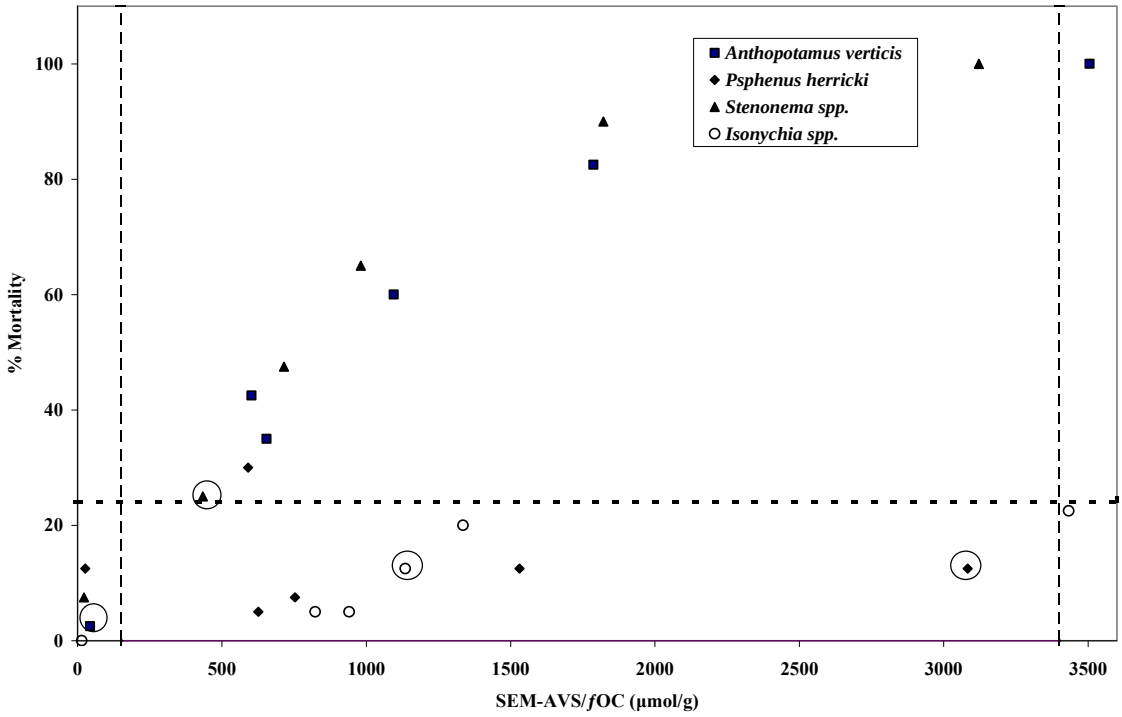

Figure 4-9. (SEM_{Ni}-AVS)/*foc* relationships with indigenous organism mortality in the Ni sediment flow-thru exposures. Vertical dashed lines represent (SEM_{Ni}-AVS)/*foc* 150 and 3400, range of uncertainty and horizontal dashed line is the 24% mortality level (Berry et al. 1996). All of the reference treatments were below (SEM_{Ni}-AVS)/*foc* < 42.7. The circles represent the Dunnett's test results for the no effect treatment levels.

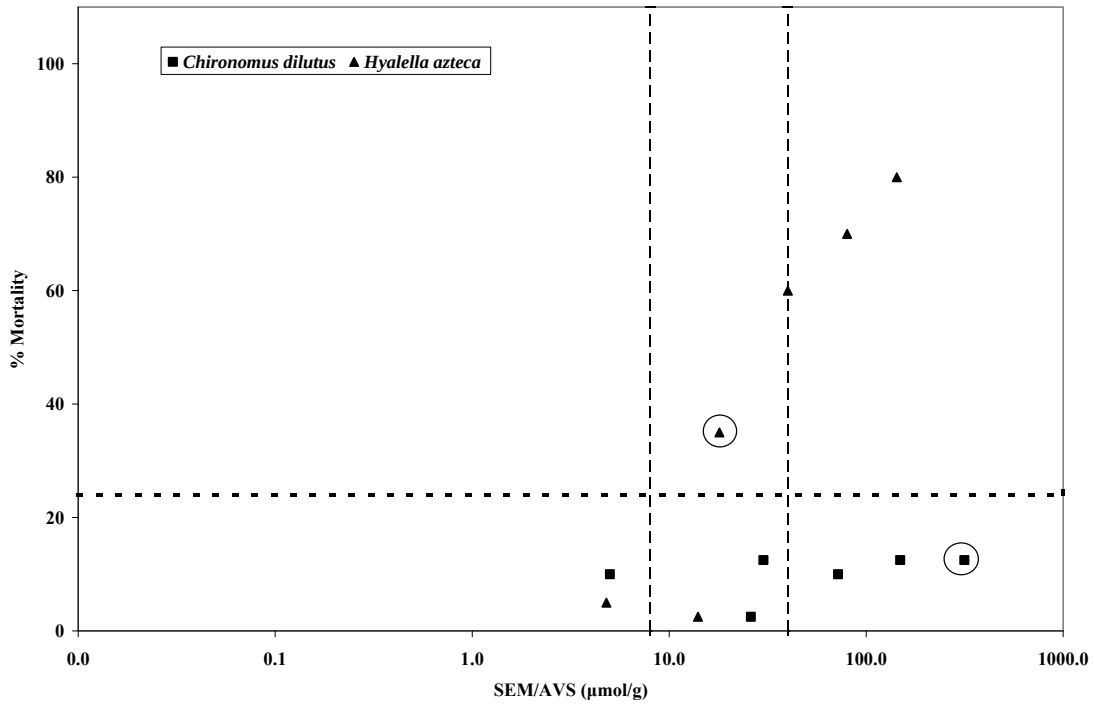

Figure 4-10. Surrogate organism mortality to log SEM_{Ni}/AVS values in the Ni sediment flow-thru exposures. Vertical lines represent SEM_{Ni}/AVS 8 and 40, range of uncertainty, and horizontal dashed line is the 24% mortality level (Berry et al. 1996). All of the reference treatments were below SEM_{Ni}/AVS < 5.0. The circles represent the Dunnett's test results for the no effect treatment levels.

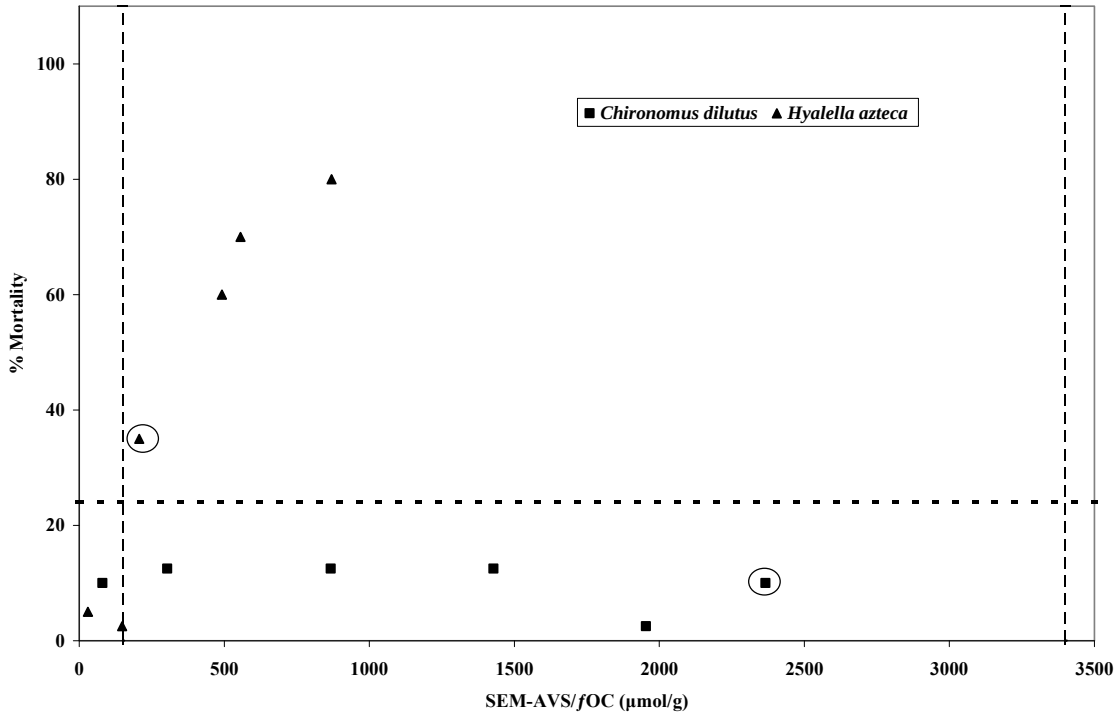

Figure 4-11. (SEM_{Ni}-AVS)/*foc* relationships with mortality data for the surrogate organisms used in the Ni sediment flow-thru exposures. Vertical lines represent (SEM_{Ni}-AVS)/*foc* 150 and 3400, range of uncertainty, and horizontal dashed line is the 24% mortality level (Berry et al. 1996). All of the reference treatments were below (SEM_{Ni}-AVS)/*foc* < 80.0. The circles represent the Dunnett's test results for the no effect treatment levels.

CHAPTER 5 – THE EFFECT OF NICKEL, SITE, AND SEDIMENT CHARACTERISTICS ON BENTHIC MACROINVERTEBRATE COMMUNITY COLONIZATION (2009)

1-0 ABSTRACT

The objectives of this study were to evaluate benthic macroinvertebrate community responses in the presence of Ni-spiked sediments, while considering the role of sediment physical characteristics. This study differs from that reported in Chapters 2 and 3 in that it used Ni-spiked sediments deployed *in situ* at three different sites, these sites varied in physico-chemical conditions, and benthic macroinvertebrate communities were allowed to colonize substrates versus being transplanted to colonize. Benthic communities were exposed to a dilution gradient of Ni ((high, low Ni, and reference concentrations) on two sediment types, Warden Ditch (WD) (high AVS, OC) and Greenville Creek (GC) (low AVS, OC). These sediments were deployed *in situ* at three sites, Greenville Creek (Ohio, USA), Warden Ditch (Ohio, USA), and Little Molasses River (LMR) (Michigan, USA). Ni flux from sediments was observed as in previous Ni studies however, Ni loss was attenuated when Ni-spiked WD sediments were deployed at the WD site. Ni loss may have been attenuated by redox changes when sediments were placed back in anoxic conditions. Taxa richness, abundance, and EPT taxa decreased with increasing SEM_{Ni} and SEM_{Ni}/AVS values at 14 and 28 d. Site differences were detected with higher richness and abundance at the GC site. The silt/clay sediments (WD) had significantly lower EPT, Ephemeroptera, Trichoptera, and abundance than the sandy/gravel sediments

(GC) at GC and LMR sites. The presence of Ni in sediments negatively affected colonization of benthic invertebrates, as did sediment type. Caged *H. azteca* exposed for 4 d after sediment deployment showed no acute or sublethal effects. Ni and sediment type interacted to affect benthic macroinvertebrate responses.

2-0 INTRODUCTION

Metals have an affinity to acid volatile sulfides (Di Toro et al. 1996), and organic carbon whether it is the dissolved or particulate phase, organic carbon can affect metal bioavailability (Gaillardet et al. 2003). USEPA (2005) states the main metal binding phases in sediments includes organic carbon, and that dissolved metals in sediments are easily adsorbed to DOC. Acid volatile sulfides are an important component of anoxic sediments, and one that can control metal bioavailability due to its ability to bind up free metals in sediments (Di Toro et al. 1996; Rickard and Morse 2005; Burton et al. 2007). In oxic freshwater sediments a common phase of organic carbon is in the particulate form, and porewater is DOC (possibly colloidal) (USEPA 2005).

Batley et al. (2002) stated that SEM/AVS model is best at predicting whether a sediment is non-toxic, but falls short in predicting toxicity because of other metal binding compartments (i.e. organic carbon). However, other studies have shown the importance of SEM/AVS ratios for determining toxicity in contaminated sediments (Di Toro et al. 1990; Berry et al. 1996; Lee et al. 2000; Burton et al. 2007). The SEM-AVS difference normalized to fraction of OC has been shown to provide a better model for predicting

toxicity in sediments (Di Toro et al. 2005). There are however, uncertainty bounds for this normalized model. The USEPA (2005) states that toxicity is possible when Σ(SEM-AVS))/foc is >3,000 µmol/gOC, and not toxic when OC concentrations are below 130 µmol/gOC and uncertainty when OC concentrations are between 130 and 3,000 µmol/gOC (USEPA 2005). When SEM/AVS >1, metals should appear in the porewater in order of highest solubilities first (i.e. Ni first) (Berry et al. 1996). These metal sulfides displace iron and manganese sulfides to form insoluble sulfides, thus making them essentially non-bioavailable (Di Toro et al. 1990). In addition, the low solubilities of metal sulfides will result in lower metal concentrations in the porewater (Simpson et al. 1998).

Ecological function of the system can be negatively affected by increased metal concentrations (Rasmussen et al. 2008), and the system can experience an ecological threshold (Clements and Rohr 2009). Metal contamination can cause benthic communities to experience thresholds of resistance where loss of benthic species can cause community shifts to more metal tolerant species, and fish energy costs can greatly increase in these contaminated systems (Pyle et al. 2005, Rasmussen et al. 2008, Clements and Rohr 2009). Here it becomes important to determine if metal concentrations in both ambient water and sediments are at concentrations high enough to cause toxicity, and are these bioavailable. Benthic macroinvertebrates provide numerous important ecological functions to stream processes (i.e. organic matter processing, particulate removal, nutrient cycling) (Vannote et al. 1980). Feeding strategies such as

shredders, collectors, scrapers, and predators help support a functioning benthic community (Vannote et al. 1980). These benthic macroinvertebrate communities are also the important to the food web, and transfer of energy to higher trophic levels. Protection of these benthic communities is vital to sustaining ecological function of aquatic ecosystems.

2-1 Objective

The objectives of this study were to evaluate benthic macroinvertebrate community responses in the presence of Ni-spiked sediments, while considering the physical characteristics of two sediment types and the three sites. Also determine if benthic invertebrate communities prefer larger grain size sediments over smaller grain size sediments by differences in diversity indices and benthic metrics.

2-2 Hypothesis: Macroinvertebrate colonization will be negatively affected by increasing Ni, and also site differences will be observed. Benthic macroinvertebrate communities will colonize larger grained substrates over smaller grained substrates.

3-0 MATERIALS & METHODS

3-1 Field sites and experimental design

Three field sites were chosen for deployment of Ni spiked sediment trays in Ohio (Greenville Creek (GC) and Warden Ditch (WD)) and Michigan (Little Molasses River (LMR)). Greenville Creek and Warden Ditch are located in Southwest Ohio and have

very high hardness concentrations, and low dissolved organic carbon (DOC) content. Little Molasses River was selected in Michigan due to the site having lower hardness and alkalinity, and higher DOC concentrations. Greenville Creek and Warden Ditch are located in Southwest Ohio, USA and the land use is primarily agriculture. Little Molasses River is located in Northeast Michigan, USA and the land use is primarily forest.

Sediments were collected from GC and WD to examine the effects of benthic macroinvertebrate colonization on Ni amended sediments. Greenville Creek sediments are low in organic carbon (OC), acid volatile sulfides (AVS), and larger grain sizes (i.e. sand/gravel). Warden Ditch sediments are high in OC and AVS, and smaller grain sizes (i.e. clay/silt).

3-2 Sediment spiking and deployment

Sediments were spiked with two concentrations of Ni (low and high) using previous effect results (Chapters 2-4), and each sediment type had a reference treatment. The GC high (1463 μg/g, 24.9 μmol/g) and low (260 μg/g, 4.4 μmol/g) concentrations at GC and WD sites, and high (1469 μg/g, 25.0 μmol/g) and low (270 μg/g, 4.6 μmol/g) at LMR site. The WD high (6894 μg/g, 117.7 μmol/g) and low (495 μg/g, 8.4 μmol/g) concentrations at GC and WD sites, and high (7363 μg/g, 125.4 μmol/g) and low (757 μg/g, 12.9 μmol/g) at LMR site. The Ni concentrations were chosen based on effects

observed from chapters 2-4. Sediment moisture content, Ni spiking, and equilibration methodology followed the methods described in Chapter 2, sec 3-3.

In the field all Ni spiked sediments were loaded into mesh lined trays (25 cm L x 7.6 cm W x 6.3 cm H) as used in Chapters 2 and 3. Four trays were deployed for each treatment (i.e. low Ni, high Ni, and reference). Sediment trays were then placed in mesh laundry bags to help sediments from eroding out of the trays during high flow events.

The trays were then deployed onto the existing stream substrate, and the trays were arranged with top being flush with the existing sediments, and then anchored with spikes and plastic ties (Fig 5-1). The GC and WD sediments were deployed on 24-Aug-09, and LMR site was deployed on 25-Aug-09. Sediments were retrieved at 2 and 4 wk intervals, on 7-Sept-09 and 21-Sept-09 for GC and WD, and on 8-Sept-09 and 22-Sept-09 for LMR.

3-3 Sediment sampling for sediment chemical characterization and benthic organisms

Two trays (replicates) from each treatment per sediment type were retrieved from each stream at 14 d and 28 d intervals. Methodology for sampling and storing sediment chemistry and processing benthic organisms is described in Chapter 2, sec 3-6.

Total sediment metal digestions are performed in Teflon digestion vessels. Dried sediments are added to each vessel along with concentrated HNO_3 and HCl acid (3:2 volume) (USEPA 2007). Solutions are then analyzed on a Flame Atomic Absorption Spectrophotometer for total Ni, Fe, and Mn. The AVS and SEM_{Ni} were determined

following USEPA (1991) AVS method, and the abbreviated method from Chapter 4, sec 3-7. Sediment % solids are determined by drying samples of sediments for 24 h at 100°C. Sediment dry weight for total organic carbon content was determined by loss on ignition (LOI) at 550°C for 1 h (Dean 1974, Heiri et al 2001). All sediment chemical concentrations are presented as concentration on a dry weight basis.

3-4 Physico-chemical and sediment pH monitoring

Stream physico-chemical parameters dissolved oxygen, conductivity, temperature, pH, hardness, alkalinity, DOC, total water Ni, and sediment pH were taken at each deployment and retrieval.

All Ni samples were filtered through an acid-clean 0.45 μm mesh filter. Samples were stored in clear 50 ml acid-cleaned centrifuge tube containers, and preserved with HNO_3 to a pH of 2. All DOC samples were filtered (0.45 μm) and preserved with HNO_3 to a pH of 2 and stored in 40 ml opaque containers. All DOC samples were analyzed on an Apollo 9000 Combustion TOC Analyzer, with standards, blanks, and duplicates for QA/QC.

3-5 In situ toxicity testing

Inoculation of dried red maple leafs (*Acer rubrum*) was conducted at the University of Michigan by lab personnel. Fungus (*Cladosporium sp.)* inoculation of *A. rubrum* involved letting leaves leach for four days in running tap water at 12°C, then

drying for two days at 40°C. Leaves were then stored in polyethylene bags at room temperature until needed. When leaves were needed they were rehydrated in Huron River, Mi, USA stream water. Leaf discs were cut into 1 cm diameters, and then sterilized in a 1 L flask with 250ml stream water in an autoclave. After cooling, the flask was inoculated with a fungus, and incubated for 10 days or longer at room temperature. Leaf discs were then dried at 60°C for 48 h and weighed to the nearest 0.01 mg.

The *in situ* toxicity testing chambers used at the sites were adapted from chambers used in Burton et al. (2005a). Each chamber had 250 µm nylon mesh windows to facilitate water and sediment exchange while deployed on top of the sediment trays. The treatments received three replicate chambers which were placed against the sediment, and three replicate chambers which were placed in the water column. Each replicate received 10 leaf discs, and 10 *H. azteca*, and were exposed for 96 h starting on 24-Aug-09 for GC and WD, and 25-Aug-09 for LMR. Lab controls were run for the duration of all *in situ* tests, and survival was 96 ± 7 %.

In situ testing ended after 96 h, and all *H. azteca* were counted and recorded for survival, and the leaf discs were carefully removed with featherweight forceps, rinsed with DI water, and placed in 50 ml centrifuge tubes. Leaf discs were then dried at 60°C for 24 h, weighed to the nearest 0.01 mg, and reported as leaf disc loss/replicate. The LMR chambers were retrieved and counted by University of Michigan personnel, and counted and processed in the same manner.

3-7 Data analysis

Data analyses were performed in the same manner as described in Chapter 3, sec 3-8. 'A host of benthic metrics (e.g. number of EPT, % EPT, % Ephemeroptera) and diversity indices (e.g. Shannon Diversity Index, Pielou's J, Simpson's Index) were calculated with a total of 39 such indices and metrics being used. All statistical analyses were run on SAS 9.2 or Minitab 16.

A multiple linear regression was used to determine relationships between the dependent variables (metrics and indices) and independent variables (sediment and water chemical variables). Sediment and water chemical variables were ln + 1 transformed, count data was square root + 0.5 transformed, and proportional data was arcsine transformed following recommendations in Zar (1999). The terms site (GC, WD, LMR), date, and sediment type (GC or WD) were used as categorical variables.

A step-wise regression with backward elimination was used to determine term(s) selection in the model. When regression models were found to be significant ($\alpha < 0.05$), multicollinearity was determined by using a variance inflation factors (VIF) criteria of < 4 to determine if terms were collinear (Pan and Jackson 2008; O'Brien 2007). A test for Heteroscedasticity (Chi-square $\alpha < 0.05$) was also used for model selection, and then an interaction term was added and analyzed again with the following criteria (Zar 1999).

Sediment chemistry assumptions described in Chapter 2 (section 3-9) were also followed in this study. Experimental replicates were collected for each sediment type and treatment; however, analytical replicates on these treatments were not performed on the

individual sediment trays. The sediment chemistry variables (total Ni, total Fe, total Mn, SEM_{Ni}, AVS, and TOC), and the SEM_{Ni}/AVS models used in the regression models were all assumed to be similar from the replicated tray. Since only two trays were collected at each time point, one tray was frozen for SEM_{Ni}/AVS, and the other stored at 4°C for total metals and TOC. The abbreviated SEM_{Ni}/AVS method described in Chapter 4, sec 3-7 was followed, and AVS concentrations were assumed for all GC samples, and used for statistical analyses and SEM_{Ni}/AVS models. It is important to understand these replicate and AVS assumptions can eliminate variance between replicates, and these approaches could possibly overestimate or underestimate any regression models and subsequent models of bioavailability. However, due to experimental and logistical constraints these assumptions were necessary to follow.

For all *in situ* toxicity testing results, the same statistical methods and assumption tests were used as described in Chapter 2 (section 3-9). Any replicated data presented in tables and graphs are means ± standard deviations.

One-way ANOVA with Dunnett's test was used on selected benthic metrics to determine no effect levels for the SEM_{Ni}/AVS models. The WD or GC reference values were used to calculate the control values.

Testing for colonization differences between GC and WD reference sediments was performed with a two-sample *t*-test, and assumption of normality was tested using the Kolomogrov-Smirnov test of residuals. If normality was violated then non-parametric Kruskal-Wallis was used. Significance was determined at $\alpha < 0.05$.

4-0 RESULTS AND DISSCUSSION

4-1 Sediment chemistry and bioavailability

There were distinct differences in Ni, AVS, Total Fe, and TOC in WD vs. GC sediments at all three sites (GC, WD, and LMR) (Tables 5-1 – 5-3). The WD sediments were spiked at 117 μmol/g (6894 μg/g) and 125 μmol/g (7363 μg/g) at WD/GC and LMR, respectively (Tables 5-1 – 5-3). The GC sediments were spiked at 24.9 μmol/g (1463 μg/g) and 25.0 μmol/g (1469 μg/g) at WD/GC and LMR, respectively (Tables 5-1 – 5-3). Ni levels were aligned with past studies where effects were observed in hardwater streams (Warden Ditch and Stillwater River) (Chapters 2, 3).

WD sediments had high levels of AVS, Fe, and TOC, and GC sediments had markedly lower levels of these sediment chemical variables (Tables 5-1 – 5-3). AVS differences were observed between the sediment types as found in Chapters 2 and 3. AVS ranges for WD sediments were 0.21-5.01 μmol/g, and GC sediments were 0.03-0.07 μmol/g (Tables 5-1 – 5-3). Total Fe also was markedly different between the two sediment types, but did not vary between sites. Total Fe ranges for WD sediments were 246-331 μmol/g, and GC sediments ranged from 102-487 μmol/g (Tables 5-1 – 5-3). The GC sediments had large range of total Mn (6-23 μmol/g), where WD sediments were had a tight range of Total Mn at the three sites (6-8 μmol/g) (Tables 5-1 – 5-3).

Ni flux was observed in both sediment types, but GC sediments lost more total Ni over time than did WD sediments (Table 5-4). At 28 d collection, the GC sediment high

Ni treatments at GC 82%, and at the LMR site these high Ni treatments 75% (Table 5-4). The high Ni treatments in WD sediments at GC and LMR lost 60% and 54%, respectively (Table 5-4). GC and WD sediments at all sites lost significant amounts of Ni over time, however WD sediments deployed at WD did not have Ni fluxing from sediments like the GC sediments deployed at this site (Tables 5-2, 5-4). WD sediments at WD site only showed a ~2% loss during the 28 d (Table 5-4). TOC losses were observed at 14 and 28 d in GC Ni treatments (Tables 5-1 – 5-3), and the same trend of increasing TOC content with increasing Ni treatments as seen in Chapters 3 and 4. All of the Ni treatments showed TOC gains at LMR, and increased with increasing Ni (Table 5-3).

This study showed similar Ni loss at all sites over time, and these results were comparable to the findings reported in Chapters 2-4 and studies by Boothman et al. (2001) and Naylor et al. (2006). The Ni losses in this study appear to be a function of spiking methods and equilibration times, and improved spiking methods have been developed by Costello et al. (2011). Liber et al. (2011) has stated that there is no consensus on equilibration times for spiking sediments with metals (i.e. Ni). However, a clear difference in Ni flux was observed at WD site. The WD Ni-spiked sediments had the lowest % change results between the three sites. The WD sediments highest Ni concentration only lost ~2% Ni over the 28 d exposure. In contrast, WD sediments lost 52% and 73% at LMR and GC, respectively. The substrate at GC and LMR was predominantly sand, gravel, and cobbles. These surrounding substrates most likely allowed oxic conditions to persist, and affected the WD sediment redox. Miao et al.

(2006) stated that disruption of anoxic sediment by oxic conditions, changes redox conditions, and creates an environment for metal flux. Simpson et al. (1998) has suggested that physical changes to sediments have the potential to release metals under oxidation events. When the WD sediments were transplanted back into similar substrates found at WD, this likely allowed for anoxic conditions to persist, and Ni flux was attenuated at 28 d. Also changing over time in WD was AVS (Table 5-4). At all three sites (WD, GC, LMR) the SEM/AVS values increased with time, however, (SEM_{Ni}-AVS)/foc values did not change as markedly, mainly because sediment OC were not changing. As AVS values decrease and sediments become oxidized, Ni bioavailability could increase (USEPA 2005) and these metals could become toxic or scavenged by other ligands (Miao et al. 2006).

Based on these results, further investigation into Ni flux is warranted when field transplanting Ni-spiked sediments. Ni flux may not solely be a function of spiking methods, but rather from redox changes due to loading, transplanting, and site redox conditions. These types of oxic conditions were seen in both Chapters 2-4 studies, and during this study at GC and LMR sites.

4-2 Sediment pH and site physico-chemical differences

Sediment pH declined with sediment depth in both GC and WD sediments at all sites (Table 5-5). The WD sediments had lower pH readings (6.49-7.22) than GC sediments (6.84-7.71) at all sites (Table 5-5). Hardness, alkalinity, and DOC were

similar at GC and WD sites. However, GC (344±14 mg/L of $CaCO_3$) and WD (374±9 mg/L of $CaCO_3$) hardness concentrations were ~3x higher than LMR hardness (116±6 mg/L of $CaCO_3$) concentrations. The LMR DOC (6.6±1.9 mg/L) concentrations were ~2-3x higher than GC (3.1±0.4 mg/L) and WD (2.1±0.7 mg/L) sites (Table 5-5).

Di Toro et al. (2005) stated that the sediment biotic ligand model (sBLM) would benefit from an increase in sediment pH data. The sediment pH values followed a similar trend as observed in MR and WD (Chapter 2), GC and BC (Chapter 3), and GC (Chapter 4) which showed a decreasing pH with increasing Ni concentration. The anoxic sediments types (WD, BC) were always showing lower pH than the more oxic sediments (GC, MR). These changes in sediment pH are expected with the addition of metals, and are being affected by hydrolysis and oxidation of the sediments (Simpson et al. 2004).

4-3 In situ toxicity testing

In situ toxicity testing with *Hyalella azteca* showed no acute toxicity from Ni during a 96 h exposure at WD or GC. *H. azteca* survivals at WD on both sediments were > 96%, and at GC on both sediments were > 88%. Results from LMR were inconsistent because of low retrieval of reference organisms (GC sediments 53% and WD sediments 77%), and subsequent higher survival in all other Ni treatments. Costello et al. (2011) deployed *H. azteca* acute toxicity exposures at LMR which were concurrent with the exposure times in this study, and they found no acute Ni toxicity or feeding effects.

There were no *H. azteca* feeding effects detected any of the sites These results were similar to Chapter 2 and 3 *in situ* toxicity tests, which showed no acute toxicity at GC and WD from Ni flux into overlying waters.

4-4. Benthic community responses

Site and benthic colonization with increasing Ni

Benthic macroinvertebrate communities responded to increasing bioavailable Ni (SEM_{Ni}/AVS models), lower DOC, increasing SEM_{Ni}, and site (GC, WD, LMR) differences (Tables 5-6, 5-7). The number of EPT taxa declined with increasing SEM_{Ni}/AVS values (Table 5-7, and Fig 5-2). Ephemeroptera composition (%) increased with increasing (SEM_{Ni}-AVS)/foc (Table 5-7, and Fig 5-3). Macroinvertebrate abundance declined with increasing SEM_{Ni} and DOC, and with decreasing AVS (Table 5-6 and Fig 5-4). Taxa richness also responded negatively to increasing SEM_{Ni} concentrations (Fig 5-5). For graphical purposes only, taxa richness was plotted against (SEM_{Ni}-AVS)/foc, and shows a negative relationship with decreasing taxa richness as (SEM_{Ni}-AVS)/foc values increase (Fig 5-6). The metrics % EPT and number of Ephemeroptera taxa both showed a negative relationship to site (WD), increasing hardness (WD), and substrate type (WD) (Table 5-6). Single taxa responses such as Hydropsychiidae and Heptageniidae both had low or absent numbers at sites WD and LMR, and populations were higher at GC site (Table 5-6, 5-7). The % Burrowers showed an increase as SEM_{Ni} increased (Table 5-6). Hardness effects were observed in numerous

benthic metrics however, results are confounded by WD sediments having the highest hardness and lowest abundance and diversity.

EPT Taxa number was negatively related to the SEM_{Ni}/AVS model, and demonstrated that EPT taxa are sensitive to bioavailable Ni in the spiked sediments, and mainly on GC sediments. These data are similar to the results in Chapter 3, which showed % EPT taxa were decreasing with increasing SEM_{Ni}/AVS values. This study also showed EPT taxa were sensitive to bioavailable Ni, and had negative relationships to increasing SEM_{Ni}/AVS values. Costello et al. (2011) found that the SEM_{Ni} had a negative relationship with total abundance, and the results from this study also showed the SEM_{Ni} demonstrating a negative relationship with total abundance. Costello et al. (2011) also demonstrated decreasing taxa richness with increasing (SEM_{Ni}-AVS)/foc values, and this study also showed a similar trend, however was not significant.

The % Ephemeroptera results stand out because these showed a positive relationship with increasing (SEM_{Ni}-AVS)/foc values. This suggests that Ephemeroptera taxa (Family level) are increasing with increasing (SEM_{Ni}-AVS)/foc values. The % Ephemeroptera increases were a result of increasing Baetidae with increasing Ni and (SEM_{Ni}-AVS)/foc on GC sediment at GC site at 28 d. However, numbers of Heptageniidae, *Isonychia spp*., Baetiscidae, and Leptophybidae all decreased with increasing Ni and (SEM_{Ni}-AVS)/foc on GC sediments at GC site. Also, Leptophlebiidae decreased with increasing Ni and (SEM_{Ni}-AVS)/foc at LMR site on GC sediments. Baetidae numbers were the most abundant of the mayflies sampled, and this family may

be exhibiting a higher tolerance to Ni than the other Ephemeroptera taxa sampled. Barbour et al. (1999) has suggested that Baetidae family is tolerant of organic pollution, and abundances generally increase as organic pollution increases. Baetidae numbers were increasing with increasing Ni, and could suggest that this family is tolerant to Ni. Results from Chapter 4 have shown that *Stenonema spp.* (Heptageniidae) is moderately sensitive to Ni on GC type sediments in a laboratory flow-thru design.

Site differences were observed in the EPT metrics (number of EPT taxa, % EPT Taxa, number of Ephemeroptera, and % Ephemeroptera Taxa) which was demonstrating the higher EPT diversity found at GC site over LMR and WD sites. There was an absence of EPT taxa found at WD on both sediment types, and low numbers of EPT taxa found at LMR. Substrate type (WD) was also negatively related to % EPT Taxa, number of EPT Taxa, and number Ephemeroptera and is explained by the low numbers of individuals found on WD substrates versus the GC substrates. This trend was similar at all three sites tested. The increase of % Burrowers with increasing SEM_{Ni} was being affected by increasing numbers of chironomids at 28 d on WD sediments at all sites. Also contributing to this increase in % Burrowers were increasing Sialidae numbers WD site. Chironomids and Sialidae are organisms that prefer fine grained sediments (silt) which are indicative of WD sediments (Voshell 2002). The variation between sediment types in this study was similar to the results observed in Chapter 2, where sediment type was an important predictor of macroinvertebrate colonization.

The DOC concentrations were higher at LMR site (6.6 ± 1.9 mg/L), compared to WD (2.1 ± 0.7) and GC (3.1 ± 0.4). Lower hardness and alkalinity suggests Ni at LMR would be more bioavailable, but with higher DOC levels would complex Ni. Evidence of hardness protection has been seen in numerous studies (Meyer et al. 1999; Pyle et al. 2002; Keithly et al. 2004). DOC is also an important ligand affecting Ni bioavailability (Di Toro et al. 2001, 2005; Doig and Liber 2006). Cloran et al. (2010) found DOC concentrations (≤ 18 mg/L) improved *D. magna* survival in Ni tests. The benthic responses showed negative responses to increasing DOC and increasing hardness (Tables 5-6, 5-7). However, WD site had the lowest abundance and diversity, but had the highest hardness. Examining benthic community responses at GC and LMR, benthic responses were negatively affected by lower hardness and increasing DOC concentrations. This would suggest that hardness is playing an important role in benthic responses to Ni on colonization trays.

Theoretical no effect (not toxic) levels of SEM/AVS models have been defined (USEPA 2005, Di Toro et al. 2005), but recent investigators (Burton et al. 2005, Burton et al. 2007, Nguyen et al. 2010, Costello et al. 2011) have demonstrated SEM/AVS no effect levels have deviated from theoretical values. In this study, the no effect levels for the three SEM/AVS models for GC sediments at 14 d at GC site were: $SEM_{Ni}/AVS <$ 20.8 µmol/g, $(SEM_{Ni}\text{-}AVS)/foc <$ 171.9 µmol/g, and $SEM_{Ni}\text{-}AVS <$ 0.92 µmol/g. Number of EPT taxa no effect levels for GC 14 d sediments at LMR of $SEM_{Ni}/AVS <$ 28.9 µmol/g, $(SEM_{Ni}\text{-}AVS)/foc <$ 221.6 µmol/g, and $SEM_{Ni}\text{-}AVS$ was 1.5 µmol/g.

However, WD sediments at GC site were not significant, and no effect levels could be predicted. The WD sediments had variable and low numbers of EPT taxa, and this contributed to non-significant values.

The SEM/AVS models are best at predicting when sediments will not be toxic; however recent research has shown that these no effect levels are deviating from these traditional values (Burton et al. 2005, Nguyen et al. 2011, Costello et al. 2011, Chapters 2-4). In Chapter 3, the number of EPT taxa on GC sediments (14 d) showed no Ni effects levels for the three SEM_{Ni}/AVS at: SEM_{Ni}/AVS < 14.8 µmol/g, (SEM_{Ni}-AVS)/foc < 329.9 µmol/g, and SEM_{Ni}-AVS < 1.6 µmol/g. In Chapter 2, Caenidae was the only invertebrate response to show a marginal no effect level, but these were not significant (p = 0.081). In Chapter 2 Caenidae no effect levels were estimated at 13.02 µmol/g (SEM_{Ni}-AVS), and 230.9 µmol/g (SEM_{Ni}/AVS). The no effect levels were consistent with other studies (Burton et al. 2005, Burton et al. 2007, Nguyen et al. 2011) which showed increasing SEM_{Ni}/AVS and SEM_{Ni}-AVS model predictions over the theoretical no effect levels (USEPA 2005). Costello et al. (2011) stated that taxa richness and Shannon diversity no effects fell within the theoretical guidelines for SEM_{Ni}/AVS < 1 and (SEM_{Ni}-AVS)/foc, respectively. In this study, SEM_{Ni}/AVS for number of EPT taxa showed no effect of 20.8-28.9 µmol/g, and (SEM_{Ni}-AVS)/foc of 171.9-226.1 µmol/g. These values are higher than the theoretical no effect thresholds of SEM/AVS < 1, and (SEM-AVS)/foc < 130 µmol/g (USEPA 2005), or SEM/AVS < 9 and (SEM-AVS)/foc < 150 µmol/g as reported by Burton et al. (2007). However, using the values reported in

Burton et al. (2007) of SEM-AVS < 2, all values were below the no effect level, but were higher than SEM-AVS ≤ 0 (USEPA 2005). All SEM/AVS models were in agreement that no effect threshold values were exceeding theoretical values (Di Toro et al. 2005, USEPA 2005). One explanation is that GC sediments are much lower in AVS and TOC, and the Ni bioavailability is assumed to be higher. Lack of these important ligands, lends these sediments to have more bioavailable porewater Ni compared to WD type sediments. Lack of WD SEM/AVS threshold data is demonstrating a benthic community sediment preference of GC sediments.

Benthic macroinvertebrate sediment preference

Benthic metrics were tested to determine if GC reference sediments had increased macroinvertebrate colonization (diversity, benthic metrics) versus macroinvertebrate colonizing WD sediments, at all three sites. Benthic metrics showed significant differences ($p \leq 0.05$) with four metrics (abundance, % Trichoptera, % Ephemeroptera, % EPT) (Table 5-8). Abundance and % Trichoptera showed a preference for GC sediments over WD sediments (Table 5-8, Fig 5-7). The % Ephemeroptera and % EPT preferred GC sediments over WD sediments at the LMR site (Table 5-8). The WD site did not show any differences in benthic communities colonizing the different sediment types. There were a host of benthic metrics which were marginally significant at $\alpha \leq 0.08$, which is suggesting that GC sediments were being preferred (Table 5-8). The %

Predators metric ($p = 0.057$) at LMR had increased percentages on WD sediments vs. GC sediments (Fig 5-8).

The results from macroinvertebrate preference of GC reference sediments vs. WD reference sediments showed that sediment type is driving benthic colonization responses when tested side by side at the same sites. At GC site, abundance and % Trichoptera both demonstrated a significant increase on GC sediments. The GC site has been listed as an Exceptional Warmwater Habitat designation by the Ohio EPA (OEPA 2000), and has a high diversity of benthic macroinvertebrates. Beisel et al. (1998) found that macroinvertebrate richness increased with increasing substrate heterogeneity (i.e. increasing pebbles, cobbles, boulders). Benthic metrics which were not significant ($\alpha \leq 0.08$) at GC and LMR sites are suggesting further investigation into GC sediment type preference. Although these results are above $\alpha = 0.05$ cutoff, these are low *p*-values and suggest further investigation. The % Predators and % Tolerant differences at LMR is due to the overall low sample size of invertebrates sampled on WD sediments. The sample sizes at all sites (GC, WD, LMR) on GC sediment treatments (reference and Ni) were commonly $\geq 50\%$ more than those found on WD sediments.

The GC sediments which were deployed at WD site had higher invertebrate numbers than WD sediments however, these were not significantly different. All of the GC sediment trays were covered with a fine layer of silt/clay upon retrieval at the WD site. Minshall and Minshall (1977) found an increase of Chironomids when they placed coarse substrate filled trays within fine sediments (pool area). They also found that fine

sediments were depositing on top of the coarse substrate trays in these pool areas (Minshall and Minshall 1977). The Minshall and Minshall (1977) results are similar to the results from this study, where GC sediments (coarse) placed in WD sediments (fine) did not produce a statistical increase in diversity or abundance. Kochersberger et al. (2012) found that coarse sediments deployed in riffle areas were experiencing sedimentation, but did not affect macroinvertebrate colonization.

5-0 GENERAL CONCLUSIONS

The objectives of this study were to evaluate benthic macroinvertebrate community responses in the presence of Ni-spiked sediments at the three sites, and also to determine if benthic invertebrate communities prefer larger grain size sediments over smaller grain size sediments. These objectives were achieved by showing the relationships between benthic community responses to increasing bioavailable Ni (SEM_{Ni}/AVS), site differences (GC), and substrate preference (larger grained GC sediments). The hypotheses were supported which demonstrated that benthic communities were responding to increasing Ni, and were preferring large grained substrates over fine-grained substrates. These results are similar to the results presented in Chapters 2-4, which is demonstrating negative benthic effects in the presence of increasing bioavailable Ni.

Total taxa, abundance, and number of EPT taxa showed decreasing numbers with increasing SEM_{Ni} and SEM_{Ni}/AVS values after 14 and 28 d. Site (GC, WD, and LMR)

played an important part in colonization differences, with the GC site having highest taxa richness and total abundance among all sites. The WD site experienced the lowest taxa richness and abundance, and this site is not conducive to EPT taxa with its silt/clay composition. Macroinvertebrate communities responded negatively with lower hardness and higher DOC. The WD colonization effects when compared to GC sediments at the three sites. However, site comparisons have to be considered carefully, due to complexity in community diversity and abundances across spatial boundaries. The WD sediments showed lower % EPT taxa, % Ephemeroptera, % Trichoptera and abundance, and host of other benthic metrics showed marginal effects.

Ni flux has been observed in all Ni studies in this dissertation (Chapters 2-4), and was observed in the current study. Ni loss appeared to be attenuated when Ni-spiked WD sediments were deployed *in situ* at the WD site. This Ni loss appears to have been slowed due to limiting oxidation and physical changes to WD sediments by placing these sediments back in similar anoxic conditions. This trend warrants further investigation in Ni flux among anoxic sediments.

The objectives of this study and hypotheses were achieved by the above benthic community responses to increasing bioavailable Ni (SEM_{Ni}/AVS), site differences (GC), and substrate preference (larger grained GC sediments). Benthic macroinvertebrates exposed to Ni were having a negative effect community richness and diversity, and these results are similar to the results presented in Chapters 2-4. These results have shown that natural benthic colonization was affected by increasing Ni and sediment type preference.

The shifts in benthic community structure suggest that the presence of Ni could degrade the benthic community. Management of contaminated sediments lists degraded benthic structure as a beneficial use, and if impaired, could warrant further investigation in sediments contaminated with Ni. These benthic effects could have trophic level implications with disruption of energy transfer, and possible loss of species.

Table 5-1. Sediment chemistry data from the 28 d Ni colonization study for Greenville Creek and Warden Ditch sediments at Greenville Creek (Ohio) site.

Ni Treatment		SEM_{Ni}/AVS	(SEMNi-AVS)/*foc*	(SEM_{Ni}-AVS)	Total Ni	Total Ni	SEM_{Ni}	AVS	Total Mn	Total Fe	TOC	DOC
Level	Date	(umol/g)	(umol/g)	(umol/g)	(mg/kg)	(µmol/g)	(µmol/g)	(µmol/g)	(µmol/g)	(µmol/g)	(%)	(mg/L)
GC-ref-day0-GC	24-Aug-09	2.445	11.90	0.10	25	0.43	0.2	0.07	9	184	0.87	3.00
GC-260-day0-GC	24-Aug-09	73.369	426.85	3.76	260	4.43	3.8	0.05	7	109	0.88	3.00
GC-1463-day0-GC	24-Aug-09	608.904	1117.52	25.56	1463	24.92	25.6	0.04	6	129	2.29	3.00
GC-ref-14d-GC	7-Sep-09	3.429	17.52	0.12	19	0.33	0.2	0.05	7	121	0.69	2.70
GC-260-14d-GC	7-Sep-09	20.762	171.94	0.92	128	2.18	1.0	0.05	23	237	0.54	2.70
GC-1463-14d-GC	7-Sep-09	65.289	617.96	2.81	317	5.40	2.9	0.04	7	121	0.45	2.70
GC-ref-28d-GC	21-Sep-09	6.226	32.84	0.15	21	0.36	0.2	0.03	8	162	0.4	3.51
GC-260-28d-GC	21-Sep-09	24.120	140.61	0.94	43	0.73	1.0	0.04	9	136	0.67	3.51
GC-1463-28d-GC	21-Sep-09	79.506	550.93	3.67	257	4.38	3.7	0.05	7	118	0.67	3.51
WD-ref-day0-GC	24-Aug-09	0.149	-39.03	-2.17	32	0.54	0.4	2.54	7	267	5.55	3.00
WD-495-day0-GC	24-Aug-09	1.917	59.27	3.80	495	8.44	8.0	4.15	7	308	6.42	3.00
WD-6894-day0-GC	24-Aug-09	14.652	444.10	34.60	6894	117.44	37.1	2.53	7	289	7.79	3.00
WD-ref-14d-GC	7-Sep-09	0.109	-62.69	-3.58	29	0.50	0.4	4.01	7	268	5.71	2.70
WD-495-14d-GC	7-Sep-09	0.517	-23.52	-1.42	439	7.47	1.5	2.94	7	301	6.0	2.70
WD-6894-14d-GC	7-Sep-09	104.950	1160.51	73.79	1848	31.48	74.5	0.71	7	331	6.36	2.70
WD-ref-28d-GC	21-Sep-09	0.106	-56.98	-3.25	29	0.50	0.4	3.63	8	287	5.70	3.51
WD-495-28d-GC	21-Sep-09	5.290	149.97	8.95	39	0.67	11.0	2.09	7	301	5.96	3.51
WD-6894-28d-GC	21-Sep-09	137.517	466.00	29.35	2783	47.42	29.6	0.21	7	283	6.30	3.51

Table 5-2. Sediment chemistry data from the 28 d Ni colonization study for Greenville Creek and Warden Ditch sediments at Warden Ditch (Ohio) site.

Ni Treatment		SEM_{Ni}/AVS	(SEMNi-AVS)/*foc*	(SEM_{Ni}-AVS)	Total Ni	Total Ni	SEM_{Ni}	AVS	Total Mn	Total Fe	TOC	DOC
Level	Date	(umol/g)	(umol/g)	(umol/g)	(mg/kg)	(µmol/g)	(µmol/g)	(µmol/g)	(µmol/g)	(µmol/g)	(%)	(mg/L)
GC-ref-day0-WD	24-Aug-09	2.445	11.90	0.10	25	0.43	0.2	0.07	9	184	0.87	1.24
GC-260-day0-WD	24-Aug-09	73.369	426.85	3.76	260	4.43	3.8	0.05	7	109	0.88	1.24
GC-1463-day0-WD	24-Aug-09	608.904	1117.52	25.56	1463	24.92	25.6	0.04	6	129	2.29	1.24
GC-ref-14d-WD	7-Sep-09	3.293	13.98	0.11	23	0.39	0.2	0.05	13	162	0.82	2.25
GC-260-14d-WD	7-Sep-09	35.047	505.99	1.49	80	1.35	1.5	0.04	9	150	0.29	2.25
GC-1463-14d-WD	7-Sep-09	106.442	601.83	4.93	308	5.25	5.0	0.05	10	180	0.82	2.25
GC-ref-28d-WD	21-Sep-09	7.838	30.94	0.17	25	0.43	0.2	0.02	7	135	0.6	2.77
GC-260-28d-WD	21-Sep-09	63.847	329.03	2.29	162	2.75	2.3	0.04	8	109	0.70	2.77
GC-1463-28d-WD	21-Sep-09	87.255	433.09	4.42	577	9.83	4.5	0.05	16	171	1.02	2.77
WD-ref-day0-WD	24-Aug-09	0.149	-39.03	-2.17	32	0.54	0.4	2.54	7	267	5.55	1.24
WD-495-day0-WD	24-Aug-09	1.917	59.27	3.80	495	8.44	8.0	4.15	7	308	6.42	1.24
WD-6894-day0-WD	24-Aug-09	14.652	444.10	34.60	6894	117.44	37.1	2.53	7	289	7.79	1.24
WD-ref-14d-WD	7-Sep-09	0.135	-37.32	-2.21	28	0.47	0.3	2.56	7	262	5.93	2.25
WD-495-14d-WD	7-Sep-09	10.427	209.97	13.31	611	10.41	14.7	1.41	7	288	6.3	2.25
WD-6894-14d-WD	7-Sep-09	25.419	308.48	19.89	6790	115.67	20.7	0.81	6	286	6.45	2.25
WD-ref-28d-WD	21-Sep-09	0.006	-85.00	-4.98	30	0.55	0.0	5.01	7	270	5.86	2.77
WD-495-28d-WD	21-Sep-09	3.710	75.33	4.74	634	15.42	6.5	1.75	7	289	6.29	2.77
WD-6894-28d-WD	21-Sep-09	134.838	1475.78	92.96	6724	113.78	93.7	0.69	6	249	6.30	2.77

Table 5-3. Sediment chemistry data from the 28 d Ni colonization study for Greenville Creek and Warden Ditch sediments at Little Molasses River (Michigan) site.

Ni Treatment		SEM_{Ni}/AVS	(SEMNi-AVS)/*foc*	(SEM_{Ni}-AVS)	Total Ni	Total Ni	SEM_{Ni}	AVS	Total Mn	Total Fe	TOC	DOC
Level	Date	(umol/g)	(umol/g)	(umol/g)	(mg/kg)	(µmol/g)	(µmol/g)	(µmol/g)	(µmol/g)	(µmol/g)	(%)	(mg/L)
GC-ref-day0-LMR	24-Aug-09	2.878	21.21	0.12	24	0.41	0.2	0.07	12	115	0.59	5.70
GC-270-day0-LMR	24-Aug-09	56.708	554.31	3.09	270	4.60	3.1	0.06	10	176	0.56	5.70
GC-1469-day0-LMR	24-Aug-09	288.276	1070.01	21.81	1469	25.02	21.9	0.08	7	123	2.04	5.70
GC-ref-14d-LMR	8-Sep-09	4.289	20.01	0.13	32	0.54	0.2	0.04	13	487	0.67	5.07
GC-270-14d-LMR	8-Sep-09	28.838	221.58	1.48	107	1.82	1.5	0.05	6	102	0.67	5.07
GC-1469-14d-LMR	8-Sep-09	44.638	368.06	2.72	353	6.02	2.8	0.06	6	124	0.74	5.07
GC-ref-28d-LMR	22-Sep-09	11.127	17.58	0.13	23	0.39	0.1	0.01	8	150	0.7	9.06
GC-270-28d-LMR	22-Sep-09	41.767	287.72	1.95	113	1.92	2.0	0.05	7	134	0.68	9.06
GC-1469-28d-LMR	22-Sep-09	64.053	388.98	2.98	374	6.37	3.0	0.05	7	162	0.77	9.06
WD-ref-day0-LMR	24-Aug-09	0.166	-42.70	-2.25	28	0.48	0.4	2.70	7	308	5.28	5.70
WD-757-day0-LMR	24-Aug-09	1.976	61.87	3.47	757	12.89	7.0	3.55	7	311	5.60	5.70
WD-7363-day0-LMR	24-Aug-09	56.848	613.32	39.11	7363	125.43	39.8	0.70	7	328	6.38	5.70
WD-ref-14d-LMR	8-Sep-09	0.162	-38.00	-2.02	25	0.42	0.4	2.42	6	267	5.33	5.07
WD-757-14d-LMR	8-Sep-09	5.240	123.19	7.26	660	11.24	9.0	1.71	7	289	5.9	5.07
WD-7363-14d-LMR	8-Sep-09	155.525	1212.97	68.11	3505	59.72	68.5	0.44	6	290	5.61	5.07
WD-ref-28d-LMR	22-Sep-09	0.154	-51.18	-2.69	23	0.39	0.5	3.18	7	267	5.27	9.06
WD-757-28d-LMR	22-Sep-09	6.373	222.29	10.21	733	12.49	12.1	1.90	8	246	4.59	9.06
WD-7363-28d-LMR	22-Sep-09	97.453	414.55	22.40	3417	58.22	22.6	0.23	7	262	5.40	9.06

Table 5-4. Total Ni, SEM_{Ni}, AVS, total Mn and Fe, and TOC percent change for three sites (Greenville Creek, Warden Ditch and Little Molasses) in the Ni colonization study.

	% change Total Ni (14d/day 0)	% change Total Ni (28d/day 0)	% change SEMNi (14d/day 0)	% change SEMNi (28d/day 0)	% change AVS (14d/day 0)	% change AVS (28d/day 0)	% change Total Mn (14 d/day 0)	% change Total Mn (28d/day 0)	% change Total Fe (14 d/day 0)	% change Total Fe (28d/day 0)	% change TOC (14d/day 0)	% change TOC (28d/day 0)
GC-ref-day0-GC	-23	-16	-3	-1	-31	-61	-18	-13	-34	-12	-21	-49
GC-260-day0-GC	-51	-83	-75	-74	-10	-22	253	34	118	25	-39	-24
GC-1463-day0-GC	-78	-82	-89	-85	4	11	13	13	-6	-8	-80	-71
WD-ref-day0-GC	-7	-7	15	2	58	43	-5	7	0	7	3	3
WD-495-day0-GC	-11	-92	-81	39	-29	-50	-5	-4	-2	-2	-6	-7
WD-6894-day0-GC	-73	-60	101	-20	-72	-92	-6	-5	14	-2	-18	-19
GC-ref-day0-LMR	31	-6	-9	-27	-39	-81	2	-34	322	30	13	22
GC-270-day0-LMR	-60	-58	-51	-36	-4	-14	-41	-24	-42	-24	20	22
GC-1469-day0-LMR	-76	-75	-87	-86	-18	-38	-9	1	1	31	-64	-62
WD-ref-day0-LMR	-12	-20	-12	9	-11	18	-10	2	-13	-13	1	0
WD-757-day0-LMR	-13	-3	28	73	-52	-46	-2	15	-7	-21	5	-18
WD-7363-day0-LMR	-52	-54	72	-43	-37	-67	-17	-7	-12	-20	-12	-15
GC-ref-day0-WD	-9	-1	-7	11	-31	-65	51	-18	-12	-26	-6	-37
GC-260-day0-WD	-69	-38	-60	-39	-16	-30	32	17	38	0	-67	-21
GC-1463-day0-WD	-79	-61	-81	-83	11	22	50	155	39	32	-64	-55
WD-ref-day0-WD	-12	-5	-9	-93	0	97	-2	0	-2	1	7	6
WD-495-day0-WD	23	28	85	-18	-66	-58	-5	-3	-7	-6	-1	-2
WD-6894-day0-WD	-2	-2	-44	152	-68	-73	-14	-13	-1	-14	-17	-19

Table 5-5. Physico-chemical (temperature, dissolved oxygen, conductivity, pH, hardness, and alkalinity) measurement means for all sites GC, WD, and LMR during the Ni colonization study 2009. Sediment pH and temperature means are presented for each treatment level/sediment type at each site.

Warden Ditch (Stream)	Mean	St.dev	Greenville Creek (Stream)	Mean	St.dev	Little Molasses (Stream)	Mean	St.dev
Temperature (°C)	18.1	1.4	Temperature (°C)	22.3	1.7	Temperature (°C)	15.9	1.0
Dissolved Oxygen (mg/L)	7.1	0.5	Dissolved Oxygen (mg/L)	8.8	0.4	Dissolved Oxygen (mg/L)	9.2	1.0
Conductivity (µS/cm)	778	41	Conductivity (µS/cm)	681	46	Conductivity (µS/cm)	179	72
pH	7.22	0.18	pH	7.85	0.16	pH	7.50	0.53
Hardness mg/L of $CaCO_3$	374	9	Hardness mg/L of $CaCO_3$	344	14	Hardness mg/L of $CaCO_3$	116	6
Alkalinity mg/L of $CaCO_3$	301	3	Alkalinity mg/L of $CaCO_3$	241	13	Alkalinity mg/L of $CaCO_3$	104	4
DOC (mg/L)	2.1	0.7	DOC (mg/L)	3.1	0.4	DOC (mg/L)	6.6	1.9
Warden Ditch (Sediment Treatments)	**Mean**	**St.dev**	**Greenville Creek (Sediment Treatments)**	**Mean**	**St.dev**	**Little Molasses (Sediment Treatments)***	**Mean**	**St.dev**
GC Sed Ref			GC Sed Ref			GC Sed Ref		
Sediment pH	7.22	0.14	Sediment pH	7.71	0.33	Sediment pH	7.17	0.11
Sediment Temperature (°C)	16.9	1.2	Sediment Temperature (°C)	21.6	2.0	Sediment Temperature (°C)	14.5	0.0
GC Sed Low			GC Sed Low			GC Sed Low		
Sediment pH	7.05	0.08	Sediment pH	7.54	0.38	Sediment pH	7.16	0.03
Sediment Temperature (°C)	17.0	1.1	Sediment Temperature (°C)	21.6	2.0	Sediment Temperature (°C)	14.5	0.1
GC Sed High			GC Sed High			GC Sed High		
Sediment pH	6.84	0.20	Sediment pH	7.35	0.49	Sediment pH	7.14	0.04
Sediment Temperature (°C)	17.0	1.2	Sediment Temperature (°C)	21.6	2.0	Sediment Temperature (°C)	14.5	0.0
WD Sed Ref			WD Sed Ref			WD Sed Ref		
Sediment pH	7.09	0.10	Sediment pH	7.38	0.21	Sediment pH	7.06	0.04
Sediment Temperature (°C)	17.0	1.0	Sediment Temperature (°C)	21.6	1.9	Sediment Temperature (°C)	14.5	0.1
WD Sed Low			WD Sed Low			WD Sed Low		
Sediment pH	6.97	0.13	Sediment pH	7.28	0.22	Sediment pH	7.09	0.03
Sediment Temperature (°C)	17.0	1.0	Sediment Temperature (°C)	21.6	1.9	Sediment Temperature (°C)	14.4	0.2
WD Sed High			WD Sed High			WD Sed High		
Sediment pH	6.49	0.26	Sediment pH	6.97	0.47	Sediment pH	7.02	0.02
Sediment Temperature (°C)	17.2	1.2	Sediment Temperature (°C)	21.6	2.0	Sediment Temperature (°C)	14.5	0.1

GC = Greenville Creek
WD = Warden Ditch
Ref = Reference
Low = Lowest Nickel Concentration
High = Highest Nickel Concentration
* no initial readings taken after deployment of sediments

Table 5-6. Multiple regression analyses for benthic macroinvertebrate responses in the Ni 2009 colonization study. Models with SEM_{Ni}/AVS terms that were significant are highlighted in bold and underlined.

Response	Coefficients	Value	T	P(>t)	R-sq	F	df	P	VIF < 4
No. of EPT Taxa	Intercept	17.046	12.65	<0.0001	0.73	45.89	4, 67	<0.0001	0.000
	Site	-1.263	-11.37	<0.0001					3.555
	Substrate	-0.706	-6.90	<0.0001					1.131
	SEM/AVS	-0.072	-2.30	0.0245					1.135
	Hardness	-1.527	-8.71	<0.0001					3.547
% EPT Taxa	Intercept	397.109	11.55	<0.0001	0.71	54.67	3, 68	<0.0001	0.000
	Site	-33.580	-11.63	<0.0001					3.544
	Hardness	-41.579	-9.12	<0.0001					3.544
	Substrate	-12.950	-5.17	0.0245					1.000
No. of Ephemeroptera Taxa	Intercept	13.197	12.26	<0.0001	0.71	54.67	3, 68	<0.0001	0.000
	Site	-0.992	-10.97	<0.0001					3.544
	Substrate	-0.506	-6.46	<0.0001					1.000
	Hardness	-1.220	-8.55	<0.0001					3.544
% Ephemeroptera	Intercept	38.588	3.62	0.0006	0.70	31.16	5, 66	<0.0001	0.000
	Date	-9.365	-3.60	0.0006					1.356
	Site	-21.817	-10.66	<0.0001					2.239
	SEM-Ni	-8.506	-5.91	<0.0001					2.801
	DOC	42.852	9.13	<0.0001					2.606
	SEM-AVS/fOC	0.022	4.25	<0.0001					2.819
% Tolerant	Intercept	-269.655	-10.92	<0.0001	0.66	68.06	2, 69	<0.0001	0.000
	Site	22.741	10.40	<0.0001					2.430
	Alkalinity	45.231	11.37	<0.0001					2.430
Total Abundance	Intercept	8.129	6.58	<0.0001	0.65	25.01	5, 66	<0.0001	0.000
	DOC	-2.860	-6.89	<0.0001					2.037
	AVS	-1.418	-5.89	<0.0001					1.738
	SEM-Ni	-3.481	-7.21	<0.0001					1.384
	Site	4.033	4.64	<0.0001					1.982
	DOC*AVS*SEMNi*Site	0.247	2.69	0.0091					2.375
Total Taxa	Intercept	2.334	10.00	<0.0001	0.60	25.42	4, 67	<0.0001	0.000
	Site	-0.433	-4.98	<0.0001					1.915
	DOC	0.983	5.32	<0.0001					1.910
	SEM-Ni	-0.264	-6.67	<0.0001					1.005
	AVS	-0.443	-4.97	<0.0001					1.006
% Trichoptera	Intercept	73.549	4.50	<.0001	0.57	14.27	6, 65	<0.0001	0.000
	Date	3.016	2.41	0.019					1.018
	Site	-10.061	-6.74	<.0001					3.849
	SEM/AVS	-3.124	-4.50	<.0001					3.387
	AVS	-10.633	-5.35	<.0001					3.426
	Hardness	-9.203	-4.03	0.0001					3.610
	Date*Site*SEM/AVS*AVS*Hardness	0.039	2.09	0.0409					1.812
% Burrowers	Intercept	49.795	3.44	0.001	0.52	17.79	4, 67	<0.0001	0.000
	Date	7.333	2.07	0.0418					1.354
	Site	18.974	6.82	<.0001					2.236
	SEM-Ni	4.583	3.91	0.0002					1.005
	DOC	-40.357	-6.35	<.0001					2.584

Table 5-7. Multiple regression analyses from benthic macroinvertebrate responses in the Ni 2009 colonization study. Models with SEM_{Ni}/AVS terms that were significant are highlighted in bold and underlined.

Response	Coefficients	Value	T	P(>t)	R-sq	F	df	P	VIF < 4
% Clingers	Intercept	42.065	2.70	0.0087	0.50	22.38	3, 68	<0.0001	0.000
	Site	-11.634	-6.05	<0.0001					1.907
	Substrate	-8.309	-3.65	0.0005					1.000
	DOC	29.357	7.18	<0.0001					1.907
HBI	Intercept	2.486	21.92	<0.0001	0.48	21.01	3, 68	<0.0001	0.000
	Date	0.070	2.51	0.0146					1.351
	Site	0.148	6.79	<0.0001					2.226
	DOC	-0.378	-7.59	<0.0001					2.577
Hydropsychidae	Intercept	1.988	4.53	<0.0001	0.47	20.22	3, 68	<0.0001	0.000
	Site	-1.080	-6.57	<0.0001					1.907
	TOC	-0.254	-3.90	0.0002					1.005
	DOC	1.170	3.34	0.0014					1.913
Shannon's Diversity Index	Intercept	16.077	4.31	<.0001	0.44	17.51	3, 68	<0.0001	0.000
	SEM-Ni	-0.553	-4.55	<.0001					1.125
	Total Fe	-1.000	-2.47	0.0158					1.125
Hills Diversity Number N2	Intercept	28.039	3.89	0.0002	0.44	17.51	3, 68	<0.0001	0.000
	SEM-Ni	-1.173	-4.60	<.0001					1.125
	Total Mn	-2.719	-2.33	0.0229					1.125
Hill's Ratio E1	Intercept	-7.456	-4.11	0.0001	0.41	15.61	3, 68	<0.0001	0.000
	Site	0.692	4.54	<.0001					3.544
	Substrate	0.567	4.29	<.0001					1.000
	Hardness	1.282	5.33	<.0001					3.544
Margalef Richness	Intercept	6.155	7.70	<.0001	0.35	12.28	3, 68	<0.0001	0.000
	Site	-0.644	-2.09	0.0402					1.913
	SEM-Ni	-0.746	-5.32	<.0001					1.003
	DOC	1.745	2.67	0.0095					1.910
Heptageniidae	Intercept	2.746	4.59	<0.0001	0.32	10.77	3, 68	<0.0001	0.000
	Site	-0.333	-4.49	<0.0001					1.907
	Substrate	-0.305	-3.49	0.0009					1.000
	DOC	0.496	3.15	0.0024					1.907
Simpson's Diversity	Intercept	5.495	5.65	<.0001	0.24	7.31	3, 68	0.0003	0.000
	Date	-0.769	-3.35	0.0013					1.158
	DOC	0.758	2.53	0.0136					1.161
	SEM-AVS	0.015	3.05	0.0033					1.003

Table 5-8. Two-sample *t*-test results from GC, WD, and LMR sites comparing benthic colonization on GC and WD reference sediments at 28 d. Comparisons were made testing whether benthic macroinvertebrates preferred GC (sand/gravel) or WD (silt/clay) types when collected at 28 d. The (+) indicates increased metric scores, and (-) indicates decreased metric scores for the respective sediment type.

Treatment/Site	Benthic Metric	*p* -value	GC Sediment Preference	WD Sediment Preference
GC vs WD Reference at GC	**Total Abundance**	**0.025**	+	-
	% Trichoptera	**0.029**	+	-
	Baetidae	0.058*	+	-
	% Filterers	0.060*	+	-
	Taxa Richness	0.064*	+	-
	Chironomidae	0.066*	+	-
	Hydropsychidae	0.069*	+	-
GC vs WD Reference at WD	None			
GC vs WD Reference at LMR	**% Predators**	**0.049**	-	+
	HBI	0.055*	+	-
	Leptophlebiidae	0.055*	+	-
	% Intolerant	0.060*	+	-
	% Tolerant	0.062*	-	+
	% Collectors	0.062*	+	-
	% Chironomidae	0.066*	+	-
	% Ephemeroptera	0.072*	+	-
	% EPT Taxa	0.075*	+	-
	Hill's E1	0.077*	+	-

GC (Greenville Creek)

WD (Wardend Ditch)

LMR (Little Molasses River)

* marginally significant at $\alpha = 0.05$

Figure 5-1. Ni-spiked sediment trays deployed at Little Molasses River, (Mi, USA). Photo courtesy of D. Costello.

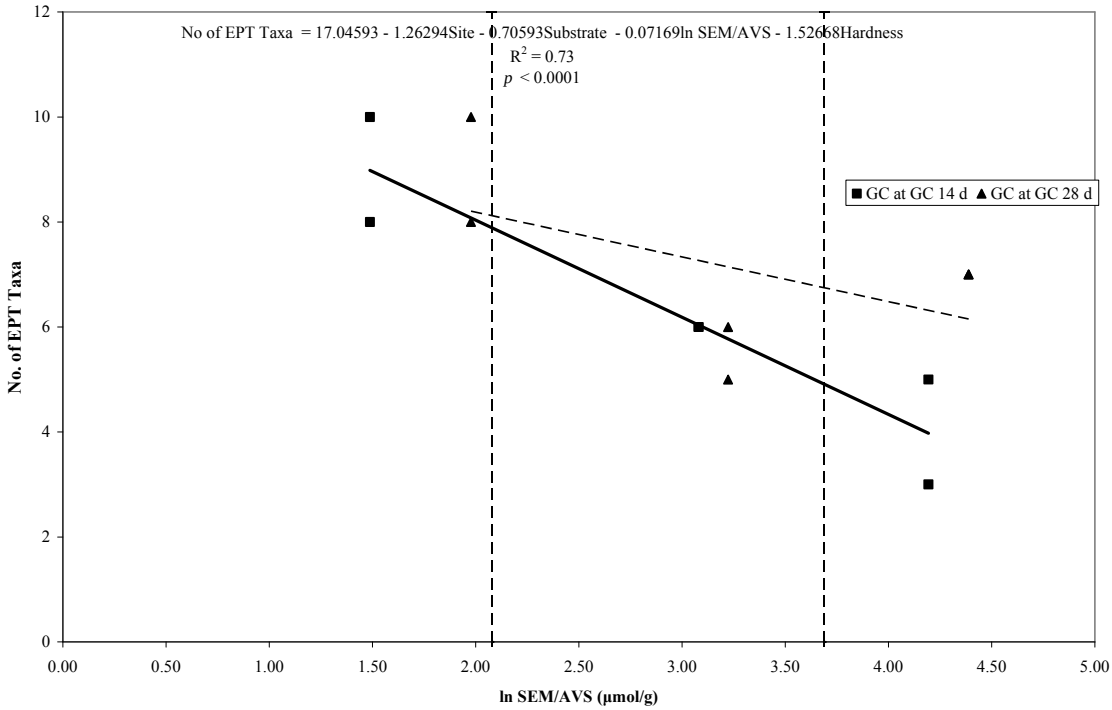

Figure 5-2. Number of EPT Taxa decreased with increasing SEM_{Ni}/AVS values in GC sediments at GC site at both 14 and 28 d. Multiple regression analysis is showing that the terms substrate, SEM_{Ni}/AVS, site and hardness are significant in the model. Vertical dashed lines represent ln values for SEM_{Ni}/AVS 8 and 40, range of uncertainty. The response number of EPT taxa are square root + 0.5 transformed, and factor SEM_{Ni}/AVS is natural log transformed. Dark regression line represents GC 14 d and dashed regression line represents GC 28 d.

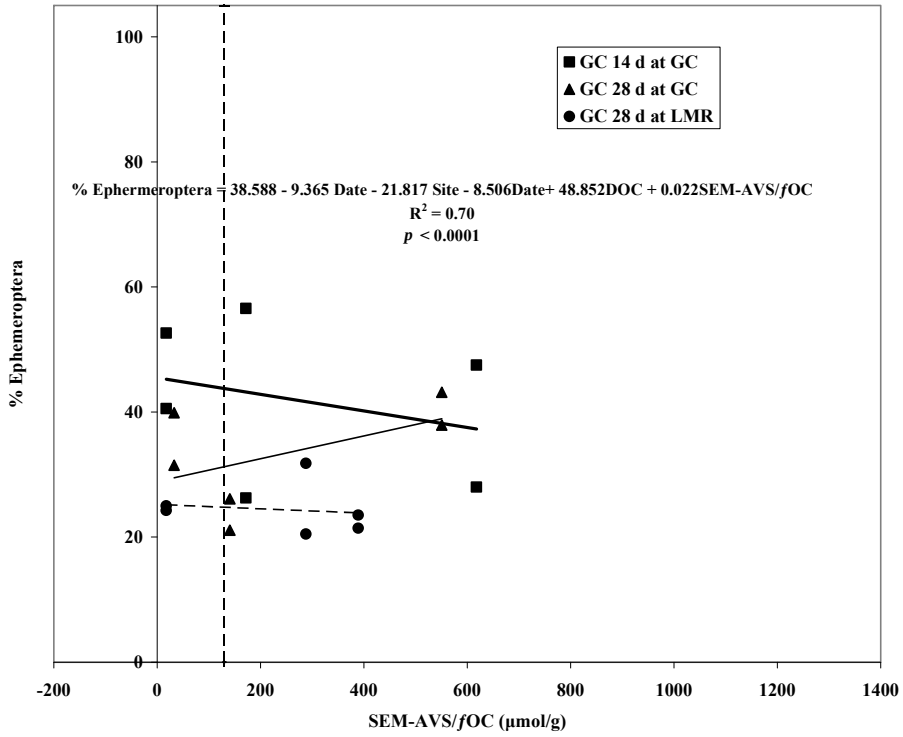

Figure 5-3. The overall model shows that % Ephemeroptera response is increasing with increasing (SEM_{Ni}-AVS)/*foc* relationships with GC sediments at GC and LMR sites. Vertical dashed line represents (SEM_{Ni}-AVS)/*foc* 130. All of the reference treatments were below (SEM_{Ni}-AVS)/*foc* < 17.5. The % Ephemeroptera Taxa was arcsine transformed, and (SEM_{Ni}-AVS)/*foc* was not transformed. Dark regression line represents GC 14 d, light regression line represents GC 28 d, and dashed line represents GC 28 d at LMR.

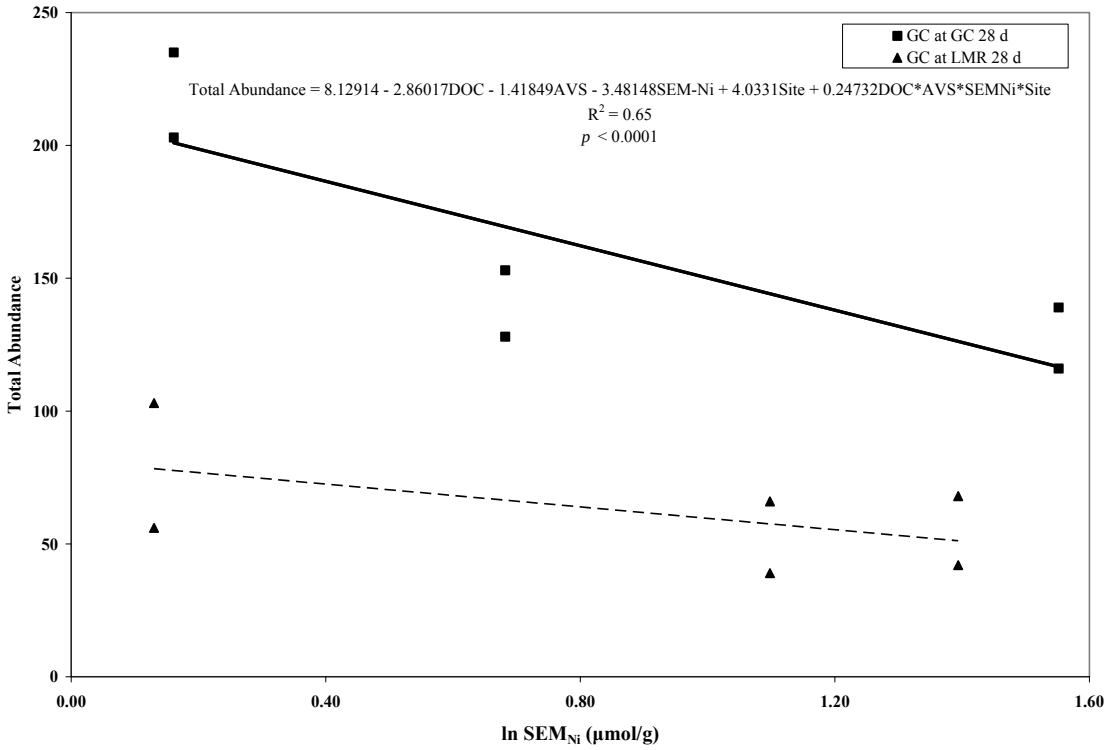

Figure 5-4. Total abundance is declining with increasing ln SEM_{Ni} on GC sediments at both sites GC and LMR at 28 d. Multiple regression analysis is showing that DOC, AVS, SEM_{Ni}, Site and the interaction term are significant in the model. The response Total abundance is square root + 0.5 transformed, and factor SEM_{Ni} is natural log transformed. Dark regression line represents GC 28 d and dashed regression line represents LMR 28 d.

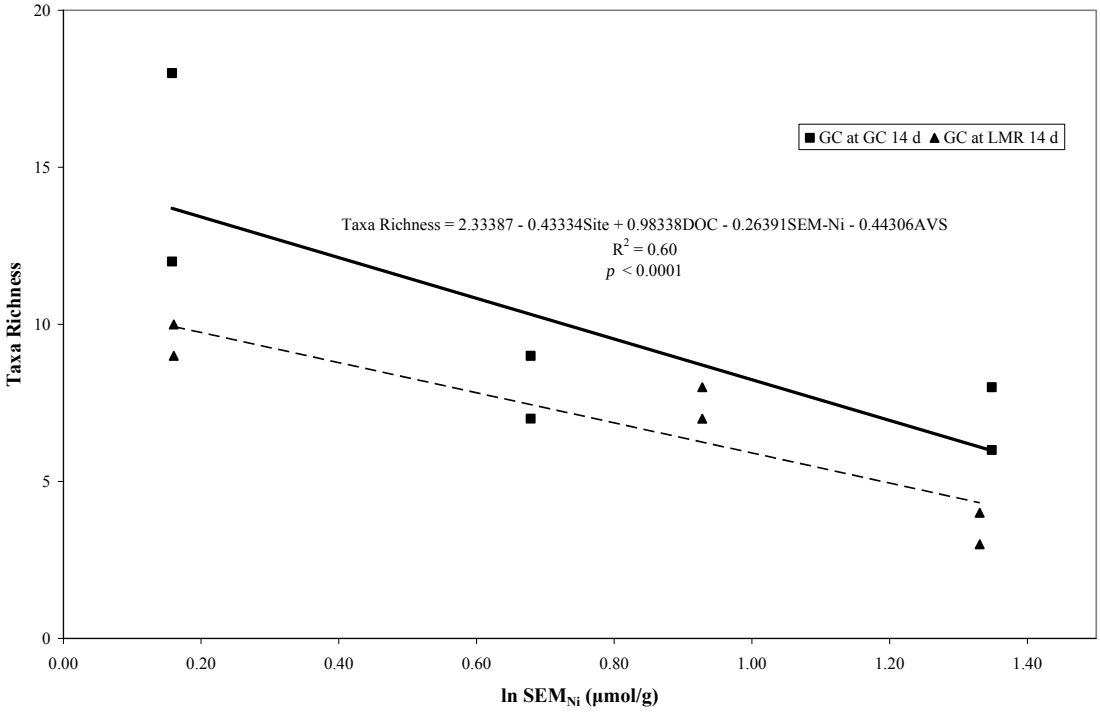

Figure 5-5. Taxa richness is declining with increasing log SEM_{Ni} on GC sediments at both sites GC and LMR at 14 d. Multiple regression analysis is showing that Site, DOC, SEM_{Ni} and AVS are significant terms in the model. Taxa richness is square root + 0.5 and SEM_{Ni} is natural log transformed. Dark regression line represents GC 14 d and dashed regression line represents LMR 14 d.

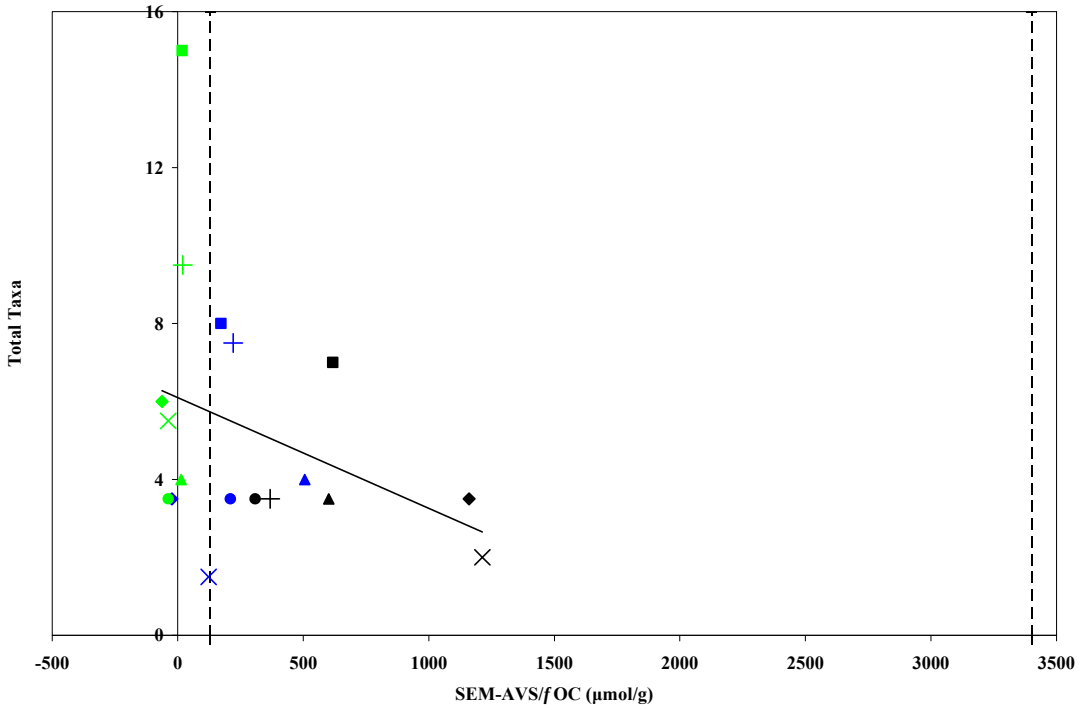

Figure 5-6. For graphical purposes, Total Taxa response was plotted against (SEM_{Ni}-AVS)/*foc* (µmol/g) at all sites (GC, LMR, and WD) at 14 d. There were no significant relationships found between the response and predictor variable, but there is a negative relationship with decreasing total taxa and increasing (SEM_{Ni}-AVS)/*foc* at all sites. Vertical dashed lines represent (SEM_{Ni}-AVS)/*foc* 130 and 3400. All of the reference treatments were below (SEM_{Ni}-AVS)/*foc* < 20.0. The regression line represents all sites at 14 d. Green symbols = reference, blue symbols = low Ni, and black symbols = high Ni. GC sediments at GC = ■, WD sediments at GC = ♦, GC sediments at WD =▲, WD sediments at WD = ●, GC sediments at LMR = +, WD sediments at LMR = X.

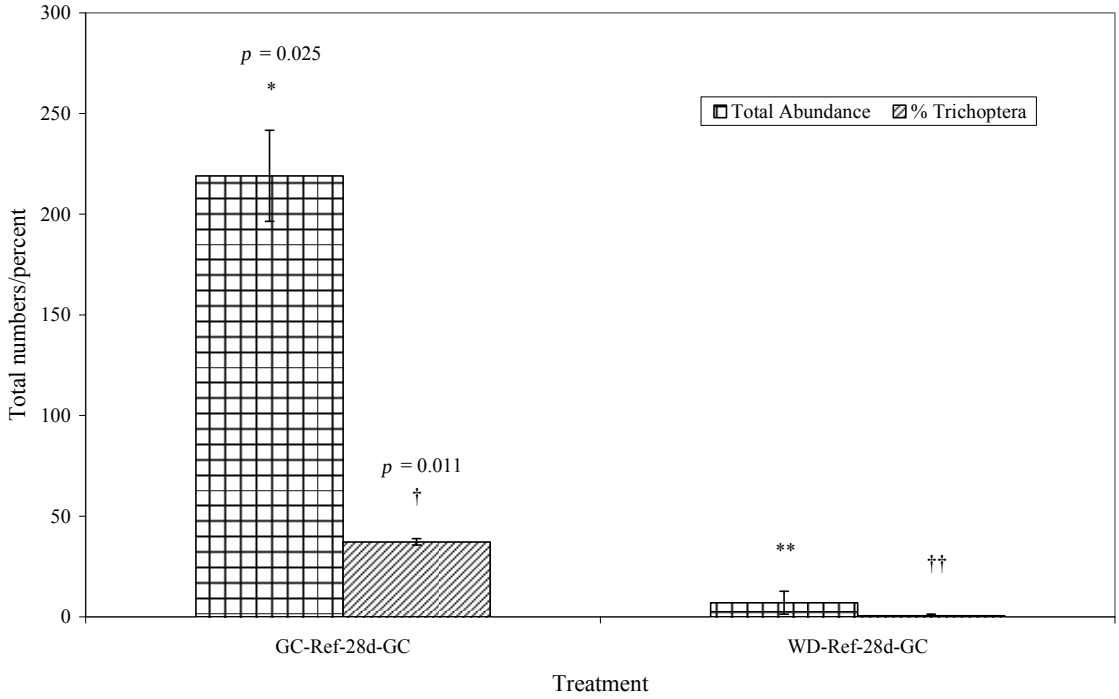

Figure 5-7. Greenville Creek (GC) versus Warden Ditch (WD) reference sediments at Greenville Creek. Total abundance and % Trichoptera had significantly higher values in GC sediments than WD sediments.

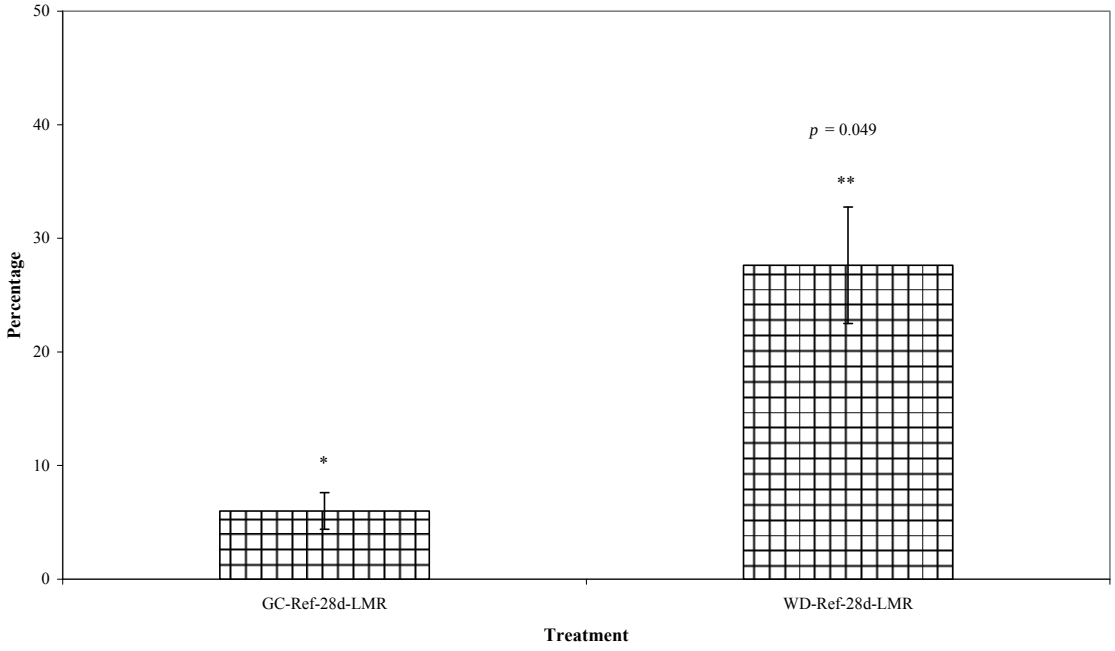

Figure 5-8. Greenville Creek (GC) versus Warden Ditch (WD) reference sediments at Little Molasses River. The metric % Predators showed increased percentages of predators on WD sediments than GC sediments.

CHAPTER 6 – LETHAL AND SUBLETHAL NICKEL TOXICITY TO *HYALLELA AZTECA* AND *LYMNAEA STAGNALIS* IS AFFECTED BY DOC, SUSPENDED SOLIDS AND THE ROUTE OF EXPOSURE (WATER COLUMN, FOOD, or SEDIMENT) (2010)

1-0 ABSTRACT

Ni bioavailability has been shown to be reduced in the presence of dissolved organic carbon (DOC), suspended solids (TSS), and other complexing ligands (AVS, OC). Organism (*H. azteca* and *L. stagnalis*) bioaccumulation of Ni has been demonstrated under food and no food experiments; however biomagnification is negligible. In this study, *H. azteca* and *Lymnaea stagnalis* were exposed to Ni amended to water, sediment, and food, either in single or combinations, while receiving dissolved organic carbon (DOC), total suspended solids (TSS), or no (Ni-only) overlying water amendments (on GC and WD sediments). In addition, food (leaf and lettuce discs) was labeled with the stable isotope of Ni (^{62}Ni), and bioaccumulation examined. Both organisms demonstrated survival, growth, and feeding inhibition effects in all Ni treatments. DOC reduced Ni effects on *L. stagnalis* survival, growth, and bioaccumulation. Ni bioavailability was highest in the GC sediments as compared to WD sediments. TSS exposures decreased survival and growth to both organisms, and increased ^{62}Ni whole body burden concentrations. Trophic transfer from food to organism was negligible (TTFs < 1.0), and the ^{62}Ni bioaccumulation was attributed to ^{62}Ni flux from food into the water. The ^{62}Ni water concentrations and ^{62}Ni-TSS exposures appeared to provide additional routes of exposure.

2-0 INTRODUCTION

The effect of multiple stressors on aquatic organisms has been an area of research that is better understood as single stressors (Breneman and Pontasch 1994; Burton 1991; Courtney and Clements 2002; Irving et al. 2003; Constable et al. 2003), but practical application to tease out multiple stressors is rarely performed (Lowell et al. 1995; Custer et al. 2006). As discussed above, Ni tends to be complexed readily by organic carbon. In addition, Sen Gupta and Bhattacharyya (2008) state that particles scavenge metals from their aqueous phase, and allows them to settle out when the particles deposit. Particle-metal interactions follow two processes, either surface adsorption or cation exchange (CEC) within the clay lattice, or even both can occur simultaneously (Abollino et al. 2008; Sen Gupta and Bhattacharyya 2008). Clays are alumniosilicates which are important components of the soil (Sen Gupta and Bhattacharyya 2008), and enter aquatic systems through runoff events from agriculture practices, or urban construction (Burton 1991).

Metals have an affinity to organic carbon whether it is the dissolved or particulate phase, organic carbon can affect metal bioavailability (Gaillardet et al. 2003). USEPA (2005) states the main metal binding phases in sediments includes organic carbon, and that dissolved metals in sediments are easily adsorbed to DOC. In oxic freshwater sediments a common phase of organic carbon is in the particulate form, and porewater is DOC (possibly colloidal) (USEPA 2005). The SEM_{Ni}-AVS difference normalized to

fraction of organic carbon (equation 1) has been shown to provide a better model for predicting toxicity in sediments (Di Toro et al. 2005). There are however, uncertainty bounds for this normalized model. The USEPA (2005) states that toxicity is possible when Σ(SEM-AVS)/foc is >3,000 μmol/g OC, and not toxic when OC concentrations are below 130 μmol/gOC and uncertainty when OC concentrations are between 130 and 3,000 μmol/gOC (USEPA 2005). Mahony et al. (1996) states that even when no AVS is present, metal concentrations may be below the sediment quality guidelines (SQG) because of the amount of OC present in the sediments.

Dissolved organic carbon (DOC) is an important ligand for metals (Ni and Cu) which has been shown to reduce metal bioavailability (De Schamphelaere and Janssen 2004; Doig and Liber 2004, 2006). However, Ni bioavailability and resulting toxicity was unaffected when tested at increasing Ni and DOC concentrations (Doig and Liber 2004, 2006). The only ameliorative effect on *H. azteca* was sublethal effects at low Ni and DOC concentrations (Doig and Liber 2006). Nickel bioavailability was unaffected by DOM source, but Ni-humic acid (HA) fractions were found in higher concentrations than fulvic acid (FA) (Doig and Liber 2004, 2006). Nickel-DOC affinities may not be as strong as other metals (i.e. Cu), and consequently Ni toxicity was less affected by DOC.

Nickel binding to Fe and its oxyhydroxide (FeOOH) in oxic sediments is another important factor affecting the toxicity. Costello et al. (2011) has suggested that metal bioavailability in sediments may be affected by increases in Fe and Mn oxides. There is a need to understand this Ni partitioning phase for use in the sediment biotic ligand

model (sBLM) but, as yet is not able to predict toxicity with confidence (Di Toro et al. 2005).

Doig and Liber (2006) stated food may complex with Ni and cause a dietary route of exposure. Wilding and Maltby (2006) have used Zn-spiked leaf discs, and shown uptake by *Gammarus spp*. Other studies have shown that metals are either adsorbing or absorbing onto food or food particles (Croteau and Luoma 2008, 2009), and these metals were readily accumulated by *L. stagnalis* during feeding tests. Croteau and Luoma (2008) have shown that *L. stagnalis* tissue content was higher in Cd, Cu, and Ni during feeding rather than water-only acute metal exposures in low hardness waters (40-100 mg/L $CaCO_3$). NiPERA has suggested dietborne Ni exposure is from food or sediment particles. They also suggest a need to discern Ni toxicity from dietary exposures vs. waterborne exposures.

2-1 Objective

The objective of this study was to demonstrate the relative importance of different Ni exposure routes to *Lymnaea stagnalis* and *Hyalella azte*ca, i.e., water, sediments, and food.

2-2 Hypothesis: Whole body ^{62}Ni accumulation from ^{62}Ni labeled food will be greater in *L. stagnalis* than *H. azteca*. The DOC and TSS amendments will not be protective of Ni toxicity or ^{62}Ni bioaccumulation during Ni sediment toxicity tests.

3-0 MATERIALS & METHODS

3-1 Laboratory experimental design

Lymnaea stagnalis and *Hyalella azteca* were exposed to a series of Ni amended compartments (Ni-water, Ni-sediments, Ni-food, Ni-all (water, sediment, food)), and each compartment had overlying water amended with TSS and DOC. Each organism, was exposed simultaneously on two sediment types: Greenville Creek (GC) (low AVS and OC), and Warden Ditch (WD) (high AVS and OC). The four Ni-amended exposures had four treatments (reference, TSS, DOC, and Ni-only), four replicates of 10 organisms (one replicate used for sediment and water chemistry), and exposed for 7 d. All *H. azteca* were 7-14 d old, and *L. stagnalis* were < 7 d post-hatch at start of each test. The Ni exposures were: (1. Ni-water) Ni-amended water + clean sediment + clean food, (2. Ni-sediment) clean water + Ni-amended sediments + clean food, (3. Ni-food) clean water + clean sediment + ^{62}Ni-amended food, (4. Ni-all) Ni-amended water + Ni-amended sediments + ^{62}Ni-amended food, and a (5. Control) clean water + clean sediment + clean food. Every exposure received overlying water changes with DOC (Aldrich Humic acid), TSS (WD sieved sediment), and nothing in the Ni-only.

Organisms were exposed in 300 ml high lip beakers with 100 ml of sediment, and ~175 ml of overlying water (Fig 6-1). Water changes (culture water, Ni-water, TSS-water, and DOC-water) were delivered (4 L) using a Zumwalt design that delivered ~ 1 L of water dispersed over all beakers twice a day. *Lymnaea stagnalis* replicates were fed

one romaine lettuce disc (17 mm in diameter), and *H. azteca* were fed three microbial conditioned *Acer rubrum* leaf discs (10 mm diameter) (as described in Chapter 5).

3-2 Ni, DOC, and TSS amendments to water and sediments

During all tests, Ni was added to the DOC, TSS, and Ni-only beakers daily, and allowed to mix in 4 L beakers on quad stir plates for a minimum of 30 min before being added to the Zumwalt system. The water in the Ni-water and Ni-all exposures was spiked at different concentrations for *H. azteca* (1603-2132 µg/L) and *L. stagnalis* (308-433 µg/L) due to differences in Ni sensitivities.

When Ni was amended to the DOC and TSS waters, the Ni was added first and allowed to mix before DOC or TSS was added. The TSS-water exposures were only for 24 h, and after 24 h the appropriate water source (culture-water, Ni-amended water) was given minus the TSS. The DOC-water and Ni-water was added for the duration of the exposure. The use of air pumps and glass pipette tips were used to suspend the TSS. All treatments received these air lines during the 24 h exposures, and after 24 h these air lines were removed and carefully examined for any organisms that may have been attached (Fig 6-1). The TSS exposures were found to be difficult to maintain constant suspension over long periods (> 2 d).

Water samples for Ni and DOC were taken on Day 1 and 7. Samples were extracted with a 50 ml syringe, and tubing was mesh lined to limit the removal of animals. The Ni and DOC samples were filtered through 0.45 µm syringe filters to

determine dissolved Ni and OC fractions. Ni and DOC samples were placed in acid-cleaned 50 ml centrifuge tubes, acidified, and stored at 4°C until analysis on Perkin Elmer Flame AA or ICPMS. DOC concentrations were analyzed on Tekmar/Teledyne TOC combustion analyzer. Ni and DOC sample QA/QC used blank and standards analyses.

The TSS samples were removed with the syringe method, and placed directly into 50 ml centrifuge tubes. These samples were later filtered through Whatman® filters for total suspended solid determination, and separate water samples were analyzed for turbidity (NTU) on an HF Scientific turbidity meter (Miami, Florida USA).

3-3 Sediment Ni spiking and sediment washing for Ni flux

The WD and GC sediments were field collected, spiked, and stored as described in Chapter 2. Both WD and GC sediments were spiked to attain similar Ni concentrations for the Ni-sediment exposures (~3.4 μmol/g, ~200 mg/kg), and Ni concentrations were lowered for the Ni-all exposures (~1.7 μmol/g, ~100 mg/kg).

Each experiment (Ni-water, Ni-sediments, and ^{62}Ni-food) had similar concentrations of Ni, and each exposure had a reference treatment. Sediment moisture content was calculated, and Ni was added to wet sediment based on dry weight calculations. Nickel ($NiCl_2 \cdot 6H_2O$ (Fisher Scientific, Pennsylvania, USA)) was introduced to sediments in 1 L wide mouth Nalgene® bottles with small volumes of

water being careful to not over-saturate the sediments. Head space in the bottles was purged with N_2 gas for 5 min before sediments were rolled for 1 h.

Spiked sediments were immediately loaded into the beakers and filled with culture water. Beakers received numerous water changes to purge the overlying water. Approximately eight water changes over 2 d were needed to flush out excess Ni that fluxed into the overlying water from the spiked sediments. The GC and WD sediments were flushed with 4 L of overlying water 2-3 times/day over a 2-4 d time period. Flushing was stopped when overlying Ni concentrations stabilized during initial tests, so as to not contribute to toxicity

3-4 ^{62}Ni food labeling

A 100 mg sample of ^{62}Ni (Oak Ridge National Laboratory (Oak Ridge, TN)) was digested with 1 ml of 16 M HNO_3, and then added to 100 mL flask with 99 mL of 100 mg/L hardness culture water. This served as the stock solution for all food labeling. The ^{62}Ni was added to both lettuce and leaf discs in two of the Ni exposures (Ni-food and Ni-all) to help discern the route of exposure for bioaccumulation. Food was submersed for 2 d in separate concentrations, 1200 µg/L (pH of 6.99-7.24) and 600 µg/L (pH of 6.95-7.30) of ^{62}Ni for the Ni-food and Ni-all exposures, respectively. The food was gently stirred daily, and after 48 h the food was rinsed with Milli-Q water to remove any ^{62}Ni not adsorbed to the food. Leaf discs (dry wt) and lettuce discs (wet wt) were weighed prior to ^{62}Ni amendments. All leaf discs were dry weights, and lettuce discs were

allowed to air dry, and any remaining moisture was blotted dry (Kim wipes), and wet weighed to nearest 0.01 mg.

Ni concentrations for the Ni-exposures

The *L. stagnalis* Ni-water test concentrations were spiked at ~450 µg/L, Ni-sediment test concentrations were ~3.4 µmol/g, Ni-food test concentrations were soaked in 1200 µg/L of ^{62}Ni, and the Ni-all test (water ~225 µg/L, sediment ~ 1.7 µmol/g, and 600 µg/L of ^{62}Ni). The *H. azteca* Ni-water test concentrations were spiked at ~2000 µg/L, Ni-sediment test concentrations were ~3.4 µmol/g, Ni-food test concentrations were soaked in 1200 µg/L of ^{62}Ni, and Ni-all test (water ~1200 µg/L, sediment ~ 1.7 µmol/g, and 600 µg/L of ^{62}Ni).

3-5 Sediment chemical characterization

An additional beaker for each treatment for chemical characterization was separated in two parts ~50 ml of sediment was placed in acid-cleaned 50 ml centrifuge tube for AVS/SEM and frozen, and the remaining 50 ml tube was stored at 4°C until digestion for total metals and TOC. Total sediment metal digestions were performed in Teflon digestion vessels. A subsample (~10 g) of each treatment was dried at 100°C for 24 h, and ~0.5 g was added to each Teflon vessel. Concentrated HNO_3 and HCl acid (3:2 ml volume) was added to each vessel followed by a series of 5 microwave heating (USEPA 2007). Vessels were then allowed to sit overnight. The next day 5 ml of digestate was diluted with 25 ml of Milli-Q water and transferred to 50 ml acid-cleaned

centrifuge tubes. Digestate was then diluted to appropriate dilution, and analyzed on a Perkin Elmer Flame Atomic Absorption or Perkin Elmer ICPMS for total Ni, Fe, and Mn. Sediment digestions for QA/QC used blanks (Milli-Q water) for every 20 samples.

The AVS and SEM_{Ni} were determined following the USEPA (1991) AVS method, and an abbreviated SEM_{Ni} extraction method (Chapter 3) was followed for GC sediments. Dried sediment total organic carbon content was determined by following methods described in Heiri et al. (2001). All sediment chemical concentrations are presented as concentration on a dry weight basis.

3-6 Physico-chemical and sediment pH monitoring

Physico-chemical parameters were monitored daily (dissolved oxygen (DO), temperature (°C), pH, conductivity (µS/cm) with an YSI 850 handheld unit. At test termination sediment pH and sediment temperature were measured by inserting the piercing tip probe (YSI pH 100 meter) directly into the sediments. Turbidity (NTU), hardness (mg/L of $CaCO_3$), alkalinity (mg/L of $CaCO_3$) were monitored at the initiation and termination of each test. Water hardness was adjusted to 100 ± 10 mg/L for all tests, using a blend of filtered well water and Milli-Q deionized water. The DOC source was Sigma Aldrich Humic acid, and TSS source was from WD sediments sieved through a 425 µM mesh, and stored at 4°C until needed.

3-7 Organism processing for growth and bioaccumulation

Depending on the test (Ni-water, Ni-sediment, Ni-food, Ni-all) a number of endpoints (survival, growth (dry weights), ^{62}Ni whole body burden, and feeding rates (leaf/lettuce loss)) were used to determine Ni effects on *L. stagnalis* and *H. azteca*. All organisms were counted at test termination (7 d) for survival, growth dry weights (weighed nearest 0.01 mg), and food (weighed nearest 0.01 mg) loss calculated as food weight at start minus food weight (weighed nearest 0.01 mg) at end of test. *Lymnaea stagnalis* survival determination was aided by viewing organism movement under a stereomicroscope. Movement inside or outside the shell was counted as surviving. Leaf and lettuce discs were recovered from the tests however, there were times when *L. stagnalis* consumed all the lettuce during the 7 d exposures. Dry weights were obtained by drying the organisms at 100 ± 2°C for 24 h and then weighing. *Lymnaea stagnalis* dry weights included shell due to the small size. Leaf discs were rinsed with DI water, dried at 100 ± 2°C for 24 h, and weighed.

In addition, ^{62}Ni whole body burden was obtained in the Ni-food and Ni-all exposures. After survival was recorded, living organisms were used for ^{62}Ni whole body content. Organisms were rinsed with 200 µmol/g of EDTA for 5 min, and rinsed with Milli-Q water (Wilding and Maltby 2002). Food and organisms were digested in acid-cleaned 15 mL centrifuge tubes with 1.3 ml of HNO_3 (Baker Instra) and 0.2 ml of H_2O_2 (30% ACS), and heated at 65 ± 5°C for 18 ± 4 h (Sola and Pratt 2006). Solutions were stored at 4°C until needed, and then diluted (10-10000x) with Milli-Q water for instrument analysis. Whole *L. stagnalis* (shell and tissue) were digested due to their

small size. Cravo et al. (2004) found that gastropod shells were not good predictors of Ni bioaccumulation. All ^{62}Ni whole body and water samples were analyzed on a Perkin Elmer ICPMS, and all sediment digestions were analyzed on a Perkin Elmer Flame AA. During digestions, blanks were used, and during instrument analyses, blanks, standards, and recalibration curves were run every 10 samples (ICPMS), and every 20 samples (Flame AA).

3-8 Data analysis

A Two-way ANOVA was used to test whether survival, growth, or feeding was affected by Ni additions in the five Ni exposures Ni-water, Ni-sediment, Ni-food, Ni-all, and Controls. All sediment, water, and food Ni responses were pooled together into a Water sediment food (WSF) term, and DOC, TSS, and None (Ni-only) were pooled together into addition (ADD) term. Organism responses in the absence of Ni (Controls) were tested in Reference, DOC, and TSS treatments, and were used as the basis for reference/control in the Two-way ANOVA analysis. When significance was determined, an interaction term (WSF:ADD) was added. One-way ANOVA with Tukey's multiple comparisons was used when the ADD term was significant. Normality and equal variance assumptions were tested using Komolgrov-Smirnov, and Levene's test, respectively. If outliers were present, the data was analyzed using a GLM procedure, and outliers removed. If the resulting *p*-value did not change from significant to non-

significant or vice versa, then data was determined to be valid, and was used for analysis in the Two-way ANOVA.

Additionally, a one-way ANOVA was used to test *L. stagnalis* and *H. azteca* survival, growth, and feeding with respect to sediment type, treatments, and Ni-exposure. Tukey's multiple comparison was used to identify treatment effects, and ANOVA assumptions were tested as described above. Results from the one-way ANOVA were presented in a matrix with a '-'(no effect) and '+' (effect).

^{62}Ni whole body burden and subsequent ratios were tested using a GLM with Tukey's pairwise comparisons. All ratios were log + 1 transformed, and residuals were tested for normality using Komolgrov-Smirnov test, and Levene's Test for equal variance. If these were violated, then a Kruskal-Wallis non-parametric test was used. All ratios of organism and food were converted to ng/g prior to statistical analyses. For ratios using Total Ni, the Total Ni denominator is these calculations was accounting for all other isotopes of Ni (58Ni, 60Ni, 61Ni, 64Ni)). Total Ni was used in Food and Water ratios, but not in trophic transfer factors (TTF).

All survival, growth, and leaf disc data is presented as mean ± standard deviation. For graphical purposes, a simple linear regression analysis was used to show relationships between ^{62}Ni whole body concentrations and ^{62}Ni water concentrations.

4-0 RESULTS AND DISCUSSION

4-1 Sediment washing and Ni flux

The WD sediments stabilized sooner than the GC sediments, but the GC sediments were never able to eliminate Ni flux completely. The GC sediments always had higher overlying Ni concentrations than WD sediments. Mean 7 d overlying Ni concentrations were < 62 µg/L in *L. stagnalis* tests, and < 36 µg/L in the *H. azteca* tests (Tables 6-1, 6-2). The overlying Ni concentrations in GC sediments at the end of the *L. stagnalis* Ni-sediment test were ~23 µg/L, and *H. azteca* Ni-sediment test ended with ~14 µg/L (Tables 6-1, 6-2). The concentrations were much lower than the growth and mortality effects seen in the Ni-water tests for *L. stagnalis* (~450 µg/L) and *H. azteca* (~2000 µg/L) during this study.

It has been hypothesized (Chapter 5) that oxic water penetrating the GC sediments (predominantly gravel/sand) are affecting Ni flux. The GC sediments have lower TOC, AVS, and Fe concentrations (Tables 6-1, 6-2), and therefore, fewer Ni binding sites. Fe and Mn oxides are most likely predominate in GC sediments, and other authors have found that in sandy-gravel sediments Fe and Mn oxides are an important partitioning phase (Sundby 1994, Chapman et al. 1998, Gomez-Alvarez et al. 2007).

4-2 DOC, TSS, and turbidity

The TSS concentrations were constant during the Ni exposures (Tables 6-1, 6-2). The *L. stagnalis* TSS concentrations in the treatments ranged from: Reference 0-2.5 mg/L, TSS treatments from 28-73 mg/L, DOC treatments from 0-5 mg/L, and Ni-only 0-7.5 mg/L (Table 6-1). The *H. azteca* TSS concentrations in the treatments ranged from:

Reference 0-2.5 mg/L, TSS treatments from 28-125 mg/L, DOC treatments from 0-5 mg/L, and Ni-only 0-5.0 mg/L (Table 6-2). Turbidity levels followed a general linear pattern with increasing TSS concentrations (Tables 6-1, 6-2). Cloran et al. (2010) found a similar linear pattern with NTU and TSS concentrations. The TSS concentrations in this study were within in the no effect levels for *Daphnia magna* as found by Cloran et al. (2010).

4-3 Sediment chemical characteristics

WD sediments have significantly higher concentrations of AVS, Total Fe, and TOC compared to GC sediments (Tables 6-1, 6-2). Both sediments had similar concentrations of Total Mn and SEM-Mn (Tables 6-1, 6-2). Ni flux out of sediments was greatly attenuated with the sediment washings, and both sediments during the Ni-amended tests showed similar Ni concentrations (Tables 6-1, 6-2). GC sediments lost more Ni (greater flux), but were also spiked with higher Ni concentrations to account Ni loss over time. The previous studies (Chs 3&5) used contrasting sediment types (high and low AVS and OC) which were distinct in their sediment chemical characterization. This study used similar sediment types with contrasting sediment chemical characterization.

4-4 Physico-chemical monitoring

Culture water hardness was ~100 mg/L, and alkalinity ~60 mg/L of $CaCO_3$. Temperature, conductivity, and pH were held constant throughout all tests and were ~22°C, ~350 µS/cm, and pH ~ 7.9, respectively. The sediment temperatures and pH readings demonstrated the similar general trends of decreasing pH with increasing Ni concentration.

These physico-chemical parameters did not appear to have extreme values that would cause stress to the organisms. The sediment temperature and pH readings were following the trend of decreasing pH with increasing Ni as reported in the previous chapters (Chs 2-5).

4-5 Lymnaea stagnalis and Hyalella azteca survival during Ni-exposures (Ni-water, Ni-sediment, Ni-Food, Ni-all)

<u>*L. stagnalis* survival on WD and GC sediments</u>

Lymnaea stagnalis showed significant survival effects ($p < 0.001$) on WD sediments with decreased survival in the Ni-water and Ni-all exposures (Fig 6-2). Ni-sediment (>93% survival) differed from (Ni-water and Ni-all), Ni-food (>97% survival) differed from (Ni-all (70-87% survival)), and Ni-water (70-97% survival)).

Lymnaea stagnalis survival decreased ($p < 0.001$) on GC sediments (Fig 6-3). There was nearly 100% mortality in all the sediment exposures (Fig. 6-3) between 2.4 - 2.8 µmol/g in GC sediments. This contrasts with > 93% survival in WD sediments at Ni concentrations of 2.9-3.3 µmol/g (Fig. 6-2). The range of $(SEM_{Ni}\text{-}AVS)/foc$ for WD

sediments was -93.0 to -64.0 μmol/g, and in GC sediments the range was from 204 to 323 μmol/g (Table 6-1).

There was a marginal DOC-amendment survival effect ($p = 0.088$) on WD sediments; suggesting that DOC may have been contributing to increased survival in the Ni-amendment tests (water, sediment, food, all), versus the Ni-only and TSS additions. A DOC protective survival effect ($p = 0.007$) in the Ni-all exposure on GC sediments. This DOC treatment demonstrated increased survival (93%) versus the Ni-only (53% survival) treatment.

Comparing *L. stagnalis* survival responses on WD and GC sediments

WD sediments were higher in TOC and AVS, and have potentially more ligands available for Ni complexation (Tables 6-1, 6-2). This is suggesting that Ni bioavailability was reduced during Ni-sediment exposures in WD sediments and *L. stagnalis* demonstrated an increase in survival during the Ni-sediment tests (Fig 6-2). Ma et al. (2010) have suggested that *L. stagnalis* are better adapted to water-only tests, and not sediment toxicity tests due to their pulmonate physiological requirements. However, *L. stagnalis* were observed interacting with the sediments throughout all the tests, and vertical migration from the sediments to the food source was frequently observed. The differences between the $(SEM_{Ni}\text{-}AVS)/foc$ are demonstrating that Ni bioavailability was increased in GC sediments, and this appears to be contributing to low survival of *L. stagnalis* during these 7 d Ni-sediment exposures. This range of the $(SEM_{Ni}\text{-}AVS)/foc$

model was below the threshold of effect in WD sediment, and in the area of uncertainty for GC sediments (USEPA 2005, Di Toro et al. 2005).

The DOC, TSS, and Ni-only amendments to water in the GC sediments showed DOC may be complexing with Ni. DOC ranged from 9.5-10.3 during all the Ni exposures (Tables 6-1, 6-2). Other studies have shown that DOC is an important ligand which can complex Ni, and reduce metal bioavailability (Playle et al. 1993, Di Toro et al. 2001, Cloran et al. 2010). Doig and Liber (2006) demonstrated DOC reduces Ni bioavailability at sublethal concentrations in *H. azteca*. Schlekat et al. (2010) and Brix et al. (2011) suggested *L. stagnalis* is highly sensitive to Ni.

L. stagnalis growth on WD and GC sediments

Lymnaea stagnalis showed increased growth effects ($p < 0.001$) on WD sediments in the Ni-sediment over the Ni-water and Ni-all exposures, and Ni-food over Ni-all (Table 6-4 and Fig 6-4). The Ni-water and Ni-all exposures experienced decreased growth compared to the Ni-sediment and Ni-food exposures on WD sediments (Table 6-4). *Lymnaea stagnalis* growth responses were ranked from highest to lowest growth: Ni-sediment > Ni-food > Ni-all > Ni-water (Table 6-4). *Lymnaea stagnalis* growth was being negatively affected by Ni in water exposures.

Lymnaea stagnalis showed increased growth effects ($p < 0.001$) on GC sediments in the Ni-food exposures over the Ni-sediment, Ni-water and Ni-all exposures (Table 6-4, and Fig 6-5). *Lymnaea stagnalis* growth responses were ranked from highest to lowest

growth: Ni-food > Ni-water > Ni-all > Ni-sediment (Table 6-4). Ni-sediment exposures were most toxic to *L. stagnalis*.

Lymnaea stagnalis growth on GC sediments did identify decreased growth in the Ni-all exposures, but the results may have been affected by smaller *L. stagnalis* starting biomass. The Ni-all had lower mean reference treatments (1.10 mg dr.wt) in the Ni-all exposures compared to the other Ni exposure reference treatments (1.70-2.57 mg dr. wt) (Table 6-4).

DOC reduced Ni toxicity in the Ni-water on WD sediments ($p = 0.003$), and in the Ni-all on GC sediments ($p = 0.008$). *Lymnaea stagnalis* growth is sensitive to Ni, and DOC is attenuating these effects on survival and growth. DOC is an important ligand which complexes with Ni, and authors have shown DOC reduced sublethal Ni toxicity (Doig and Liber 2006; Playle et al. 1993). Cloran et al. (2010) demonstrated that DOC was able to attenuate Ni toxicity on *D. magna* to a certain threshold (< 18 mg/L of DOC), and the DOC concentrations in these exposures were between 9.5-10.3 mg/L.

<u>*L. stagnalis* feeding on WD and GC sediments</u>

Lymnaea stagnalis had higher feeding ($p < 0.001$) on WD sediments in the Ni-food (^{62}Ni) exposures than other exposures. The Control and Ni-all exposures were similar in lettuce disc loss, and the Ni-water and Ni-sediment exposures showed a feeding inhibition. Ni-sediment TSS exposures (-8.57 ± 26.61 mg wet wt.) had less feeding (lettuce disc loss) than Ni-food TSS exposures (-20.67 ± 11.67 wet wt.) (Table 6-5). Also, the Ni-food TSS exposures (-20.67 ± 11.67 wet wt.) had less feeding than the DOC

(-37.83 ± 21.75 wet wt.) and Ni-only treatments (-28.57 ± 22.22 wet wt.) (Table 6-5). This is suggesting that TSS was inhibiting feeding.

Lymnaea stagnalis also had higher feeding ($p < 0.001$) on GC sediments in the Ni-food (^{62}Ni) exposures. In fact, all the relationships on WD sediments were observed on GC sediment exposures (Table 6-5). When Ni was added to all three compartments (Water, Sediment, and Food) feeding rates were negatively affected by the higher Ni concentrations.

There was a similar trend on GC and WD sediments with an increase of feeding on lettuce discs in the Ni-Food exposures with the exception of TSS interaction effect. TSS has been shown to reduce Ni toxicity (Pyle et al. 2002), but Ni-TSS exposures have also been shown to cause toxicity to a certain threshold (Cloran et al. 2010). *Lymnaea stagnalis* feeding during the Ni-water exposures in the presence of TSS showed a feeding inhibition effect. This multiple stressor interaction suggests an increase in Ni bioavailability was not the only stressor causing an inhibition of feeding. *Lymnaea stagnalis* feeding was inhibited during the Ni-water and Ni-sediment exposures on both sediment types. The increased in feeding by *L. stagnalis* on ^{62}Ni labeled food coincided with others (Croteau and Luoma 2008, 2009), and should warrant for further tests involving ^{62}Ni stable isotope and bioaccumulation work.

H. azteca survival on WD and GC sediments

Hyalella azteca survival ($p = 0.014$) decreased in the Ni-water versus Ni-food exposures (Fig 6-6) on WD sediments. No other survival effects were found in WD sediment Ni exposures.

Hyalella azteca survival increased ($p < 0.001$) in the Ni-food exposures, and the Ni-all and Ni-sediment exposures had the lowest survival (Fig 6-7) on GC sediments. The Ni-all exposures showed a strong negative relationship with Ni concentration. TSS and DOC in Ni-water exposures allowed for increased survival over Ni-sediment and Ni-all exposures. There was a synergistic effect due to decreased survival in the TSS and a significant increase in the Ni-only treatment ($p = 0.008$) (Fig 6-7).

Hyalella azteca survival showed a clear effect, and was ranked from lowest to highest survival in GC sediments: Ni-all < Ni-sediment < Ni-water < Ni-food (Fig 6-6). During the Ni-sediment and Ni-all exposures on WD sediments, survival increased in the TSS, DOC, and the Ni-only exposures compared to GC sediments (Figs 6-6, 6-7). This is suggesting that WD sediments are reducing Ni bioavailability through complexation and adsorption (Mahony et al. 1996, USEPA 2005, Di Toro et al. 2005). The range of $(SEM_{Ni}\text{-}AVS)/foc$ in the Ni-all exposures for WD sediments was -172 to -139 μmol/g, and in GC sediments the range was from 104 to 187 μmol/g (Table 6-2). Both sediment types were just at or below the predicted no effect threshold levels of the $(SEM_{Ni}\text{-}AVS)/foc$ model (Di Toro et al. 2005, USEPA 2005). The WD sediments are higher in TOC, DOC, and Fe than GC sediments (Table 6-2), and there appears to be a sediment type protective effect from Ni when exposed on the different sediments during the same

exposure time (7 d) (Figs 6-6, 6-7). The *H. azteca* TSS synergistic effect was similar to effects seen in *D. magna* in the presence of TSS and Ni (Cloran et al. 2010).

H. azteca growth on WD and GC sediments

Hyalella azteca growth was affected by Ni ($p < 0.001$) (Table 6-7) on WD sediments. *Hyalella azteca* growth results are ranked from highest to lowest: Ni-all > Ni-food > Ni-sediment > Ni-water (Table 6-7).

As in WD sediments, Ni affected growth ($p < 0.001$) (Table 6-7, Fig 6-9) on the GC sediments. There were also overall higher growth rates and were ranked from highest to lowest: Ni-food > Ni-water > Ni-all > Ni-sediment (Table 6-7).

The *H. azteca* growth on GC sediments was lower in the Ni-sediment exposures compared to WD sediments (Figs 6-8, 6-9). This trend was also observed in the Ni-all exposures and is likely a result of greater nutritional value of WD sediments. As with survival, greater effects were noted on GC sediments due to greater Ni bioavailability.

H. azteca feeding on WD and GC sediments

Hyalella azteca food consumption in WD sediments was lowest in the Ni-water and Ni-all treatments, and statistical effects ($p < 0.001$) were detected between Ni-sediment and Ni-all, and Ni-sediment and Ni-food treatments, and Ni-Food and Ni-all (Table 6-8). *Hyalella azteca* feeding was ranked from most to least feeding: Ni-sediment > Ni-food > Ni-all > Ni-water (Table 6-8).

This is suggesting that food was being avoided when Ni was amended to water in both the Ni-water and Ni-all exposures (Table 6-8). *Hyalella azteca* growth was highest

in Ni-all and Ni-food, and feeding was highest in Ni-sediment and Ni-food exposures. *Hyalella azteca* feeding was inhibited in Ni-water exposures, and growth was lowest in this exposure. In the Ni-water exposures, Doig and Liber (2006) suggest that Ni is complexing with food and causing avoidance, and results in this study are showing similar effects.

The *H. azteca* food consumption on GC sediments was lowest in the Ni-all and Ni-water (Table 6-8). *Hyalella azteca* had similar significant feeding effect as observed in the WD sediments with the Ni-sediment exposures having more feeding than the Ni-all exposures. *Hyalella azteca* was consuming more leaf disc material in the Ni-sediment exposures than the Ni-all exposures. *Hyalella azteca* feeding was ranked from most to least feeding: Ni-sediment > Ni-food > Ni-water > Ni-all (Table 6-8).

Hyalella azteca feeding inhibitions were seen in the Ni-water and Ni-all exposures on both sediment types (WD and GC). Others (Hatch and Burton 1999, Gillespie et al. 1997) have shown *H. azteca* leaf processing was negatively affected with increasing chemical concentrations. Ni-water amendments regardless of sediment type appeared to be causing feeding inhibitions, and growth effects were also observed in these Ni-water exposures.

4-6 Lymnaea stagnalis and Hyalella azteca ^{62}Ni whole body burden

Lymnaea stagnalis Ni-food in WD and GC sediments

Lymnaea stagnalis ^{62}Ni food exposures in WD sediments showed a difference in the ratio of ^{62}Ni:Total Ni in water (BCF) between Reference (2830 µg/L) and TSS (18051 µg/L) (Table 6-6), thus it appears that some ^{62}Ni fluxed from lettuce to water, and adsorbed with TSS particles (potential route of exposure). The TTFs were < 1, not showing any transfer of Ni to the next trophic level.

The *L. stagnalis* ^{62}Ni whole body burden concentrations in GC sediments were significantly different treatments. *Lymnaea stagnalis* ^{62}Ni whole body burden concentrations were ranked from lowest to highest ^{62}Ni concentrations: reference (0.5 µg/g), DOC (2.1 µg/g), TSS (13.1 µg/g), and Ni-only (13.5 µg/g) (Table 6-6).

The *L. stagnalis* ^{62}Ni organism:Total Ni Food ratio also showed an effect on GC sediments, and were ranked from lowest to highest ^{62}Ni ratio: DOC (0.00 ng/g), reference (0.01 ng/g), TSS (0.03 ng/g), and Ni-only (0.07 ng/g) (Table 6-6).

The ratio of ^{62}Ni:Total Ni in water (BCF) was significant on GC sediments, and ranked from lowest to highest ^{62}Ni BCF: Reference (1462 µg/L), DOC (2661 µg/L), Ni-only (15151 µg/L), and TSS (17586 µg/L) (Table 6-6). The TTFs were < 1 and not showing any transfer of Ni to the next trophic level.

Lymnaea stagnalis had similar ^{62}Ni whole body burden concentrations in the TSS (7.6 µg/g), DOC (8.0 µg/g), and Ni-only (6.5 µg/g) treatments on WD sediments (Table 6-6). However, it appears that ^{62}Ni amended food was not the main route of exposure in WD sediments, and appears that water and TSS exposures may have provided an

additional route of exposure (Table 6-6). The ^{62}Ni flux from food is contributing to low concentrations of ^{62}Ni in water (0.0-6.8 μg/L).

No effects were observed in the TTFs in either sediment type is suggesting little Ni is being transferred up the food chain, and thus poses a low biomagnification potential. Suedel et al. (1994) states that Trophic Transfer Coefficients (TTC) > 1 are demonstrating biomagnification has occurred, and < 1 no biomagnification is occurring.

There was an effect in ^{62}Ni whole body burden concentrations in the GC sediments, with TSS and Ni-only treatments having accumulated higher ^{62}Ni concentrations. The TSS and Ni-only treatment increase of ^{62}Ni in *L. stagnalis* showed no effect on feeding or growth (Tables 6-4, 6-5). The ^{62}Ni whole body concentrations with increasing ^{62}Ni in food is showing a linear relationship ($R^2 = 0.66$) (Fig 6-10).

The ^{62}Ni flux from food to water appears to be contributing to low concentrations of ^{62}Ni found in the water (1.3-12.1 μg/L). The BCF showed a positive linear relationship ($R^2 = 0.83$) (Fig 6-11). Deforest et al. (2007) suggested that *L. stagnalis* BAFs were demonstrating a non-negative slope, and may possibly bioaccumulate copper. The current study results are showing this similar effect, and suggesting water is a main route of exposure. On the GC sediments, TSS and Ni-only treatments showed possible routes of exposure of ^{62}Ni to *L. stagnalis*, and the DOC amendments are showing significantly lower BCF values for ^{62}Ni (Table 6-6). As found earlier, DOC is showing a protective effect from ^{62}Ni water concentrations on *L. stagnalis*. These results contrast with Croteau and Luoma (2008), which they found higher ^{62}Ni tissue concentrations from

food versus water. The results from this experiment suggest that ^{62}Ni water concentrations may be contributing more Ni whole body burden than food alone. Doig and Liber (2006) found high correlations with increasing Ni^{2+} and Ni tissue, and found low Ni levels were affected by DOC, and these results are showing a similar pattern.

Hyalella azteca Ni-food in WD and GC sediments

On the WD sediments, *H. azteca* ^{62}Ni whole body burden concentrations in Reference (1.0 µg/g), DOC (1.9 µg/g), and Ni-only (2.3 µg/g) treatments were significantly lower than the TSS (9.8 µg/g) treatment. The ratio of ^{62}Ni:Total Ni in water (BCF) was significant, and differences were between DOC (2924 µg/L)/Ni-only (3738 µg/L) and TSS (39174 µg/L) (Table 6-9). Trophic Transfer Factors (TTFs) were small < 0.02, and suggesting little Ni is being transferred up the food chain, and thus poses a low biomagnification potential

Hyalella azteca ^{62}Ni body burdens, ratios for food, water, or TTFs were not different, and showed no signs of ^{62}Ni bioaccumulation. All of the food and TTFs ratios were < 0.02, suggesting very little transfer of Ni up the food chain, and thus low biomagnification potential.

Hyalella azteca whole body burden ^{62}Ni concentrations were significantly higher in the TSS treatment on WD sediments. The ^{62}Ni:Total Ni Food and TTF ratios are all very small, and there is a negative relationship ($R^2 = 0.29$) between ^{62}Ni water and ^{62}Ni whole body burden (Fig. 6-12). This is suggesting that most of the ^{62}Ni is being

transferred to the organism from ^{62}Ni amended food (Fig. 6-13) however, this is a weak relationship ($R^2 = 0.16$). This suggests that ^{62}Ni is fluxing from food as seen in the *L. stagnalis* tests however, ^{62}Ni may have been adsorbing to TSS particles. TSS may have been an additional route of exposure, as seen in *L. stagnalis* Ni-food exposures (Tables 6-5, 6-9). Pyle et al. (2002) demonstrated that TSS was ameliorating the effects of Ni on *D. magna*, but Cloran et al. (2010) found the Ni-TSS exposures were causing effects. During this study, there was a visible layer of TSS particles settling out on the sediments and leaf discs after 24 h. The increase of ^{62}Ni *H. azteca* body burden could be explained by their shredding behavior, and subsequent consumption of TSS-^{62}Ni adsorbed particulate material.

Lymnaea stagnalis Ni-All (Ni amended food, sediment, and water) in WD and GC sediments

On WD sediments, *L. stagnalis* ^{62}Ni whole body burden concentrations showed an effect in TSS treatment (7.3 µg/g) which had the highest ^{62}Ni whole body concentrations. There were no other effects observed between any of the ratios for food, water, or TTFs, suggesting very little transfer of Ni up the food chain. The *L. stagnalis* ^{62}Ni BCF ratios for water were low, however demonstrated an inverse relationship to ^{62}Ni concentration as discussed in DeForest et al. (2007).

In the GC sediment tests, *L. stagnalis* ^{62}Ni water and TTF ratios showed effects in the Ni treatments (Table 6-10). As observed in the WD sediments, all Food ratios

were low < 0.07, but the highest TTF ratios in all the tests were found in the TSS and DOC treatments, 0.46 and 0.21, respectively. These higher TTF ratios were affected by two outliers in the TSS and DOC analyses (Figs 6-10, 6-11). If these two outliers are removed, TTF ratios for TSS and DOC fell to 0.22 and 0.09, respectively. Also, when the two outliers were removed *L. stagnalis* ^{62}Ni whole body burdens, the TSS exposures is reduced from 31.5 μg/g to 16.2 μg/g, and DOC was reduced from 20.8 μg/g to 10.5 μg/g. With the outliers removed the variability is markedly reduced, and the results are now significantly different ($p = 0.046$).

Lymnaea stagnalis exposures in the WD Ni-all had similar ^{62}Ni body burdens as the Ni-Food exposures. This is suggesting that no additional ^{62}Ni was entering the body during additional Ni amendments to the sediments and water.

Lymnaea stagnalis ^{62}Ni whole body burdens in the GC sediment during the Ni-all exposures had the highest body burden concentrations (Tables 6-6, 6-10), but no effects were detected (increased variability among replicates) (Figs 6-10, 6-11). The TTFs ratios are below the 1.0 which suggests no trophic transfer and biomagnification potential (Suedel et al. 1994). The ^{62}Ni BCF ratios for water were low (Fig 6-11), and also are demonstrating an inverse relationship to ^{62}Ni concentration as described in DeForest et al. (2007) (Tables 6-6, 6-10). This is contrasting to the results observed in *L. stagnalis* Ni-food BCF, which was showing a positive linear relationship (Fig 6-11) and suggesting Ni bioaccumulation. Adding Ni to all compartments (water, sediment, and food) appears to have affected *L. stagnalis* ability to bioaccumulate, and this would be more realistic in

natural conditions. This confirms that low Ni concentrations affecting organisms, as found in Doig and Liber (2006), are controlling Ni whole body accumulation.

Hyalella azteca Ni-All (Ni amended food, sediment, and water) in WD and GC sediments

On the WD sediments, *H. azteca* ^{62}Ni whole body burden concentrations in reference (2.0 µg/g), DOC (1.7 µg/g), TSS (7.9 µg/g), and Ni-only (4.6 µg/g) were not showing an effect. The ratio of ^{62}Ni:Total Ni in water (BCF) was significant, but no treatment effects: DOC (28 µg/L), TSS (6 µg/L), and Ni-only (14 µg/L) (Table 6-11). Trophic Transfer Factors (TTFs) were small < 0.04, and suggesting little Ni is being transferred up the food chain, and thus poses a low biomagnification potential.

The same results were seen for *H. azteca* on GC sediments. The ^{62}Ni whole body burden concentrations in reference (1.8 µg/g), DOC (3.8 µg/g), TSS (6.6 µg/g), and Ni-only (4.1 µg/g) were not showing an effect. The ratio of ^{62}Ni:Total Ni in water (BCF) was significant, and but no treatment effects: DOC (14 µg/L), TSS (20 µg/L), and Ni-only (12 µg/L) (Table 6-11). Trophic Transfer Factors (TTFs) were small < 0.03, and suggesting little Ni trophic transfer.

These results were suggesting very little accumulation of ^{62}Ni from food, and the BCF were demonstrating the negative relationship as described in Deforest et al. (2007). The growth and feeding effects observed in the Ni-all exposures were not from diet, and likely from the combination of water and sediment Ni amendments.

5-0 GENERAL CONCLUSIONS

The objective of this study was achieved, and hypotheses not completely supported. The results demonstrated that *H. azteca* and *L. stagnalis* toxicity was determined by whether Ni was amended to water, sediment, and food, and also affected by sediment type and overlying water quality (TSS and DOC). In general, Ni water was the most important route, followed by sediment, and then food. As in previous studies (Chps 2-5) sediment type has been important determinant of Ni bioavailability, and it also affected ^{62}Ni bioaccumulation in this study. DOC and TSS were protective but also enhanced to Ni toxicity and ^{62}Ni bioaccumulation, respectively. However, these responses were organism and exposure specific. Direct food transfer of ^{62}Ni was complicated due to desorption of ^{62}Ni from food to the water column, and ^{62}Ni in the water column appeared to control ^{62}Ni bioaccumulation in the organisms.

DOC did show protective Ni effects on survival and growth of *L. stagnalis* in Ni exposures. DOC is an important ligand which can affect metal bioavailability in aquatic systems (Cloran et al. 2010). TSS inhibited *L. stagnalis* feeding in Ni-water and Ni-sediment exposures. *Lymnaea stagnalis* did respond with the selected endpoints in all Ni-exposures. *Lymnaea stagnalis* was the more sensitive of the two organisms tested in this study, and Schlekat et al. (2010) and Brix et al. (2011) have identified *L. stagnalis* as one of the most sensitive organism to Ni and Cu, respectively.

Numerous *H. azteca* growth effects occurred in all Ni-exposures. An increase of Ni bioavailability to *H. azteca*, and TSS concentrations (28-50 mg/L) did show one

synergistic effect on GC sediments. *Hyalella azteca* responded to Ni with both sublethal and lethal endpoints, and WD ditch sediments providing a protective survival effect from Ni.

The results from the ^{62}Ni-food studies have demonstrated that ^{62}Ni was accumulating in both *H. azteca* and *L. stagnalis* during multiple Ni exposures with TSS and DOC water amendments on two different sediment types. Diet (^{62}Ni food) in *L. stagnalis* and *H. azteca* exposures were not contributing to adverse survival or growth effects. The differences observed in ^{62}Ni whole body bioaccumulation in the two separate exposures were complexed. The ^{62}Ni-food exposure showed an increase of ^{62}Ni in water, and this was from ^{62}Ni fluxing from ^{62}Ni labeled food. The ^{62}Ni increase in water was contributing to higher ^{62}Ni bioaccumulation in *L. stagnalis,* and TSS synergistic effect in *H. azteca*. However, in the Ni-all exposure, *L. stagnalis* bioaccumulation appeared to be more food related (higher ^{62}Ni food ratio and TTFs). This *L. stagnalis* ^{62}Ni increase may have been a function of multiple Ni exposures from the other compartments (water and sediment). Previous research has shown that *H. azteca* and *L. stagnalis* have accumulated Ni during water-only studies, and food studies (Doig and Liber 2006, Croteau and Luoma 2008). Doig and Liber (2006) have suggested that adding food during Ni toxicity tests may contribute to a dietary route of Ni exposure. Zn labeled food has been shown to bioaccumulation, and cause toxicity in lab and field aquatic organisms (Courtney and Clements 2002; Wilding and Maltby 2006). Other studies have shown that metals are either adsorbing or absorbing onto food or food

particles readily accumulated in *L. stagnalis* during feeding (Croteau and Luoma 2008, 2009). These results also showed that *L. stagnalis* metal tissue content during feeding rather than water-only acute metal exposures (Croteau and Luoma 2008). Overall, *H. azteca* and *L. stagnalis* responded similar regarding ^{62}Ni:Total Ni, food, and TTFs, and both organisms had low values, which are suggesting little ^{62}Ni transfer from food sources. The DOC amendments showed a protective effect from ^{62}Ni bioaccumulation in *L. stagnalis* in the GC sediments, and no protective effect was observed in *H. azteca*. Doig and Liber (2006) demonstrated that DOC was able to lower Ni tissue content under lower, more sublethal Ni concentrations, and suggested that Ni:DOC ratios were important to Ni-DOC complexes. Whole body burden ^{62}Ni concentrations changed little between Ni-Food and Ni-All exposures for *H. azteca*, however, *L. stagnalis* showed an increase in ^{62}Ni whole body burden GC sediments in both Ni-food and Ni-All exposures. These GC sediments have been shown to demonstrate more toxicity in Ni spiked toxicity tests (Chapters 3-5), and could be a function of limited binding sites available for free Ni.

These results suggest the compartment differences are important to understanding Ni toxicity *to H. azteca* and *L. stagnalis*. Ecotoxicologically speaking, water amended with Ni appeared to be the most important, followed by sediment, and then food. Trophic transfer of Ni was negligible, and suggests that food amended with Ni did not bioaccumulate nor manifest into toxicity of the organism. When water, sediment, and food were amended with Ni, these exposures represent realistic exposures encountered by aquatic organisms in the environment. This research has demonstrated the importance of

Ni toxicity and bioaccumulation, which can contribute data to water quality criteria and sediment quality guidelines for assisting regulatory decisions regarding Ni.

Table 6-1. Sediment and water chemistry data from all of the *Lymnaea stagnalis* 7 d Ni tests.

Date of Collection/Treatment	Test	SEM_{Ni}/AVS (µmol/g)	$(SEM_{Ni}$-AVS)/foc (µmol/g)	SEM-AVS (µmol/g)	Total Ni (mg/kg)	Total Ni (µmol/g)	SEM_{Ni} (µmol/g)	AVS (µmol/g)	Total Mn (µmol/g)	SEM_{Mn} (µmol/g)	Total Fe (µmol/g)	SEM_{Fe} (µmol/g)	% TOC	DOC (mg/L)	TSS (mg/L)	Turbidity (NTU)	Total Ni water (µg/L)
15-Apr-10 WD Ref	Ni-water	0.01	-135.6	-10.48	26.8	0.46	0.10	10.58	6.3	5.1	358	175	7.7	2.0	0.0	0.6	1.7
15-Apr-10 WD TSS		0.02	-125.8	-9.28	28.9	0.49	0.23	9.51	6.2	4.7	273	187	7.4	3.6	27.5	20.8	360.5
15-Apr-10 WD DOC		0.02	-153.0	-11.60	26.3	0.45	0.26	11.86	5.8	5.4	311	215	7.6	9.7	5.0	5.8	433.1
15-Apr-10 WD Ni only		0.03	-105.4	-7.54	30.2	0.51	0.24	7.78	6.0	5.0	282	198	7.2	2.6	7.5	0.3	412.4
15-Apr-10 GC Ref	Ni-water	0.54	-4.8	-0.04	14.1	0.24	0.04	0.08	4.9	5.3	137	18	0.8	2.0	2.5	1.2	1.1
15-Apr-10 GC TSS		0.61	-5.0	-0.03	16.8	0.29	0.05	0.08	7.2	6.1	142	20	0.6	2.4	47.5	30.8	308.1
15-Apr-10 GC DOC		0.48	-5.6	-0.04	14.4	0.25	0.04	0.08	4.8	4.3	107	19	0.7	10.0	0.0	6.1	380.7
15-Apr-10 GC Ni only		0.53	-4.3	-0.04	14.2	0.24	0.04	0.08	6.0	4.6	144	16	0.9	2.5	5.0	2.4	401.5
14-May-10 WD Ref	Ni-sediment	0.03	-116.6	-8.32	24	0.40	0.23	8.55	6.6	6.6	391	202	7.1	0.9	2.5	0.5	3.0
14-May-10 WD TSS		0.28	-65.8	-4.75	169	2.87	1.85	6.61	6.1	6.4	310	197	7.2	0.8	32.5	33.3	4.7
14-May-10 WD DOC		0.21	-93.0	-6.81	174	2.97	1.78	8.59	6.3	6.1	340	189	7.3	10.3	-2.5	4.3	2.7
14-May-10 WD Ni only		0.29	-64.0	-4.58	191	3.25	1.90	6.48	6.2	6.6	328	202	7.2	1.5	2.5	0.5	1.2
14-May-10 GC Ref	Ni-sediment	0.52	-5.2	-0.04	14	0.24	0.04	0.08	7.2	5.8	124	15	0.7	1.3	0.0	0.4	2.2
14-May-10 GC TSS		31.34	323.1	2.37	141	2.40	2.44	0.08	6.5	6.7	133	18	0.7	1.6	42.5	34.4	51.0
14-May-10 GC DOC		20.94	250.2	1.55	166	2.83	1.63	0.08	6.5	6.0	151	16	0.6	9.9	0.0	5.3	51.0
14-May-10 GC Ni Only		25.64	204.1	1.92	164	2.79	2.00	0.08	6.8	5.8	141	16	0.9	1.2	0.0	0.3	62.0
3-June-10 WD Ref	Ni-food	0.01	-177.8	-11.93	23.9	0.41	0.09	12.02	6.5	6.9	448	167	6.7	0.6	0.0	0.3	1.2
3-June-10 WD TSS		0.01	-138.8	-9.71	26.3	0.45	0.09	9.80	6.8	8.4	426	197	7.0	1.2	42.5	28.1	1.2
3-June-10 WD DOC		0.01	-243.4	-17.05	28.4	0.48	0.16	17.21	6.7	8.2	482	193	7.0	10.2	2.5	4.7	1.9
3-June-10 WD Ni only		0.02	-166.1	-11.73	26.2	0.45	0.24	11.96	6.4	8.4	402	196	7.1	0.9	5.0	0.3	3.8
3-June-10 GC Ref	Ni-food	0.52	-4.7	-0.04	16.2	0.28	0.04	0.08	7.3	6.3	245	18	0.8	0.5	2.5	0.3	1.4
3-June-10 GC TSS		0.46	-4.2	-0.04	13.8	0.24	0.04	0.08	6.2	5.6	188	16	1.0	0.8	42.5	25.8	1.2
3-June-10 GC DOC		0.47	-6.2	-0.04	13.8	0.24	0.04	0.08	6.1	5.8	139	15	0.7	9.5	0.0	4.2	2.9
3-June-10 GC Ni Only		0.54	-5.5	-0.04	18.5	0.32	0.04	0.08	6.3	7.1	210	20	0.7	0.8	2.5	0.2	2.2
23-June-10 WD Ref	Ni-all	0.03	-146.5	-9.24	28	0.48	0.25	9.49	5.9	7.5	442	192	6.3	1.2	0.0	0.3	1.4
23-June-10 WD TSS		0.07	-156.1	-9.20	133	2.26	0.66	9.87	5.9	8.4	497	174	5.9	1.0	72.5	45.6	174.5
23-June-10 WD DOC		0.08	-122.8	-7.35	121	2.05	0.66	8.01	6.0	6.9	390	173	6.0	10.1	0.0	4.0	202.7
23-June-10 WD Ni only		0.06	-169.6	-10.62	130	2.21	0.69	11.31	5.9	7.3	403	181	6.3	1.6	0.0	0.4	170.2
23-June-10 GC Ref	Ni-all	0.52	-5.4	-0.04	19	0.33	0.04	0.08	8.1	5.5	117	14	0.7	0.8	0.0	0.3	1.9
23-June-10 GC TSS		18.23	151.2	1.34	107	1.83	1.42	0.08	7.0	4.5	129	14	0.9	0.8	72.5	48.6	230.8
23-June-10 GC DOC		16.21	121.0	1.19	108	1.84	1.26	0.08	6.7	5.2	187	15	1.0	9.7	0.0	4.8	327.7
23-June-10 GC Ni Only		15.26	147.7	1.11	130	2.21	1.19	0.08	5.9	5.5	243	16	0.8	1.3	5.0	0.4	269.8
30-Oct-10 WD Ref	Controls	0.05	-54.2	-3.59	26.5	0.45	0.18	3.77	6.6	8.3	361	173	6.6	1.4	0.0	0.4	1.1
30-Oct-10 WD TSS		0.05	-54.2	-3.59	26.5	0.45	0.18	3.77	6.6	8.3	361	173	6.6	2.0	50.0	47.5	1.1
30-Oct-10WD DOC		0.05	-54.2	-3.59	26.5	0.45	0.18	3.77	6.6	8.3	361	173	6.6	11.1	0.0	5.3	1.1
30-Oct-10 GC Ref	Controls	0.81	-2.3	-0.02	16.9	0.29	0.07	0.09	7.3	7.1	151	21	0.7	1.3	0.0	0.3	0.9
30-Oct-10 GC TSS		0.81	-2.3	-0.02	16.9	0.29	0.07	0.09	7.3	7.1	151	21	0.7	3.0	50.0	45.5	0.9
30-Oct-10 GC DOC		0.81	-2.3	-0.02	16.9	0.29	0.07	0.09	7.3	7.1	151	21	0.7	11.9	0.0	5.2	1.1

WD = Warden Ditch

GC = Greenville Creek

Ref = Reference

TSS = Total Suspended Solids

DOC = Dissolved Organic Carbon

SEM = Simultaneously Extracted Metals

AVS = Acid Volatile Sulfides

Table 6-2. Sediment and water chemistry data from all of the *Hyalella azteca* 7 d Ni tests.

Date of Collection/Treatment	Test	SEM_{Ni}/AVS (µmol/g)	(SEM_{Ni}-AVS)/foc (µmol/g)	SEM-AVS (µmol/g)	Total Ni (mg/kg)	Total Ni (µmol/g)	SEM_{Ni} (µmol/g)	AVS (µmol/g)	Total Mn (µmol/g)	SEM_{Mn} (µmol/g)	Total Fe (µmol/g)	SEM_{Fe} (µmol/g)	% TOC	DOC (mg/L)	TSS (mg/L)	Turbidity (NTU)	Total Ni water (µg/L)
23-Apr-10 WD Ref	Ni-water	0.02	-166.28	-11.84	27	0.46	0.3	12.10	5.4	5.4	358	214.6	7.1	1.9	2.5	0.3	1.9
23-Apr-10 WD TSS		0.03	-151.28	-11.36	51	0.87	0.4	11.75	5.7	5.7	358	191.5	7.5	2.0	50.0	32.1	1603.9
23-Apr-10 WD DOC		0.04	-125.58	-8.67	39	0.67	0.4	9.04	5.3	5.3	276	179.5	6.9	10.5	0.0	5..8	1852.7
23-Apr-10 WD Ni only		0.03	-130.84	-9.34	45	0.77	0.2	9.58	5.6	5.6	371	189.0	7.1	3.0	0.0	0.9	1956.4
23-Apr-10 GC Ref	Ni-water	0.60	-3.36	-0.03	14	0.24	0.0	0.08	4.6	4.6	182	16.4	0.9	1.8	0.0	0.5	1.9
23-Apr-10 GC TSS		0.77	-1.50	-0.02	16	0.28	0.1	0.08	5.2	5.2	174	19.5	1.2	2.5	27.5	27.4	1842.3
23-Apr-10 GC DOC		0.88	-0.91	-0.01	14	0.24	0.1	0.08	4.9	4.9	104	16.5	1.1	9.1	0.0	5.9	1883.7
23-Apr-10 GC Ni only		0.54	-3.82	-0.04	16	0.28	0.0	0.08	3.6	3.6	151	18.8	0.9	2.2	0.0	0.6	2132.4
25-May-10 WD Ref	Ni-sediment	0.02	-59.39	-4.06	26	0.45	0.1	4.14	7.2	8.1	403	218.7	6.8	0.5	0.0	0.6	2.4
25-May-10 WD TSS		0.40	-35.81	-2.54	179	3.05	1.7	4.26	8.3	7.2	339	232.9	7.1	0.5	77.5	48.7	3.0
25-May-10 WD DOC		0.36	-39.86	-2.87	179	3.05	1.6	4.51	8.3	8.3	380	223.0	7.2	10.2	2.5	4.9	1.1
25-May-10 WD Ni only		0.38	-37.50	-2.67	191	3.25	1.6	4.31		8.3	380	181.3	7.1	0.7	-5.0	0.3	1.9
25-May-10 GC Ref	Ni-sediment	0.60	-6.06	-0.03	16	0.27	0.0	0.08	4.7	4.7	130	14.1	0.5	0.4	0.0	0.4	3.5
25-May-10 GC TSS		24.06	374.80	1.80	191.8	3.27	1.9	0.08	4.0	4.0	119	12.8	0.5	0.9	95.0	55.3	13.8
25-May-10 GC DOC		30.21	421.89	2.28	201.8	3.44	2.4	0.08	6.1	6.1	121	17.6	0.5	11.0	5.0	5.1	24.8
25-May-10 GC Ni Only		23.05	310.53	1.72	191.8	3.27	1.8	0.08	8.2	8.2	293	14.9	0.6	0.8	0.0	0.4	35.7
14-June-10 WD Ref	Ni-food	0.01	-130.96	-8.96	26	0.44	0.1	9.05	8.5	8.5	513	168.6	6.8	0.7	0.0	0.3	1.4
14-June-10 WD TSS		0.01	-216.40	-15.33	28	0.48	0.2	15.56	10.7	10.7	401	184.2	7.1	0.3	125.0	72.3	0.9
14-June-10 WD DOC		0.01	-155.18	-11.23	25	0.43	0.1	11.32	8.6	8.6	377	170.5	7.2	9.6	0.0	5.2	2.1
14-June-10 WD Ni only		0.01	-207.85	-14.14	26	0.45	0.1	14.23	9.9	9.9	327	196.5	6.8	1.6	0.0	0.4	2.1
14-June-10 GC Ref	Ni-food	0.52	-5.90	-0.04	14	0.24	0.0	0.08	4.4	4.4	108	13.8	0.6	1.3	-2.5	0.3	1.1
14-June-10 GC TSS		0.59	-7.80	-0.03	19	0.32	0.0	0.08	5.7	5.7	205	16.2	0.4	2.4	72.5	52.4	4.0
14-June-10 GC DOC		0.48	-13.48	-0.04	17	0.29	0.0	0.08	4.5	4.5	141	14.4	0.3	9.8	5.0	5.4	3.1
14-June-10 GC Ni Only		0.55	-2.78	-0.03	17	0.29	0.0	0.08	6.1	6.1	121	18.1	1.3	0.6	0.0	0.3	1.4
2-July-10 WD Ref	Ni-all	0.04	-150.34	-9.40	29	0.49	0.4	9.79	7.4	7.4	385	183.7	6.2	0.7	0.0	0.4	1.4
2-July-10 WD TSS		0.06	-172.27	-11.00	144	2.45	0.7	11.70	8.8	8.8	516	183.6	6.4	0.9	50.0	39.8	940.5
2-July-10 WD DOC		0.05	-139.67	-9.43	130	2.22	0.5	9.97	7.3	7.3	432	182.0	6.8	9.9	0.0	4.2	938.1
2-July-10 WD Ni only		0.05	-152.57	-10.07	129	2.21	0.5	10.62	8.8	8.8	395	183.0	6.6	0.8	5.0	0.5	1108.1
2-July-10 GC Ref	Ni-all	0.53	-2.99	-0.04	19	0.33	0.0	0.08	5.6	5.6	109	16.2	1.2	0.6	0.0	0.3	1.3
2-July-10 GC TSS		15.93	104.45	1.16	144	2.46	1.2	0.08	7.0	7.0	131	18.7	1.1	0.9	47.5	35.7	947.9
2-July-10 GC DOC		18.32	186.76	1.35	118	2.00	1.4	0.08	6.7	6.7	101	17.4	0.7	10.0	0.0	4.1	1147.0
2-July-10 GC Ni Only		13.87	135.06	1.00	119	2.04	1.1	0.08	5.0	5.0	167	14.6	0.7	0.8	0.0	0.5	1159.4
25-Oct-10 WD Ref	Controls	0.05	-54.19	-3.59	26	0.45	0.18	3.77	6.6	8.3	361	173.2	6.62	1.4	0.00	0.4	1.6
25-Oct-10 WD TSS		0.05	-54.19	-3.59	26	0.45	0.18	3.77	6.6	8.3	361	173.2	6.62	2.0	50.00	47.5	1.7
25-Oct-10 WD DOC		0.05	-54.19	-3.59	26	0.45	0.18	3.77	6.6	8.3	361	173.2	6.62	11.1	0.00	5.3	1.4
25-Oct-10 GC Ref	Controls	0.81	-2.29	-0.02	17	0.29	0.1	0.09	7.3	7.1	151	21.2	0.71	1.3	0.00	0.3	1.3
25-Oct-10 GC TSS		0.81	-2.29	-0.02	17	0.29	0.1	0.09	7.3	7.1	151	21.2	0.71	3.0	50.00	45.5	1.5
25-Oct-10 GC DOC		0.81	-2.29	-0.02	17	0.29	0.1	0.09	7.3	7.1	151	21.2	0.71	11.9	0.00	5.2	1.4

WD = Warden Ditch

GC = Greenville Creek

Ref = Reference

TSS = Total Suspended Solids

DOC = Dissolved Organic Carbon

SEM = Simultaneously Extracted Metals

AVS = Acid Volatile Sulfides

Table 6-3. Results from the Two-way ANOVA test, and exposure effects from Tukey's pairwise comparisons. Exposures in left columns had the highest survival, growth, or feeding rates. The corresponding + sign indicate the left column exposures have significantly higher means than the exposures in top horizontal rows.

Response	Exposure Type						
Ha WD Survival		Ni-Sediment	Ni-Food	Ni-All	Ni-Water	Controls	*p* -value
	Controls	+	-	-	-		0.014
	Ni-Food	+		-	-		
Ha WD Growth		Ni-Sediment	Ni-Food	Ni-All	Ni-Water	Controls	*p* -value
	Controls	+	+	+	+		< 0.001
	Ni-All	-	-		+		
Ha WD Feeding		Ni-Sediment	Ni-Food	Ni-All	Ni-Water	Controls	*p* -value
	Controls		+	+	+	-	< 0.001
	Ni-Sediment		+	+	+		
	Ni-Food	-		+	+		
Ha GC Survival		Ni-Sediment	Ni-Food	Ni-All	Ni-Water	Controls	*p* -value
	Controls	+	-	+	+		< 0.001
	Ni-Food	+		+	+		
	Ni-Water	+		+			
Ha GC Growth		Ni-Sediment	Ni-Food	Ni-All	Ni-Water	Controls	*p* -value
	Controls	+	+	+	+		< 0.001
	Ni-Food	+		-	-		
	Ni-Water	+		-			
Ha GC Feeding		Ni-Sediment	Ni-Food	Ni-All	Ni-Water	Controls	*p* -value
	Controls	+	+	+	+		< 0.001
	Ni-Sediment		-	+	-		
Ls WD Survival		Ni-Sediment	Ni Food	Ni-All	Ni-Water	Controls	*p* -value
	Ni-Sediment		-	+	+	+	< 0.001
	Ni-Food			+	+	-	
Ls WD Growth		Ni-Sediment	Ni-Food	Ni-All	Ni-Water	Controls	*p* -value
	Controls	-	+	+	+		< 0.001
	Ni-Sediment		-	+	+		
	Ni-Food			+	-		
Ls WD Feeding		Ni-Sediment	Ni-Food	Ni-All	Ni-Water	Controls	*p* -value
	Ni-Food	+		+	+	+	< 0.001
	Controls	+		-	+		
	Ni-All	+			+		
Ls GC Survival		Ni-Sediment	Ni-Food	Ni-All	Ni-Water	Controls	*p* -value
	Controls	+	-	-	-		< 0.001
	Ni-Food	+		-	-		
	Ni-Water	+		-			
	Ni-All	+					
Ls GC Growth		Ni-Sediment	Ni-Food	Ni-All	Ni-Water	Controls	*p* -value
	Controls	+	-	+	+		< 0.001
	Ni-Food	+		+	-		
	Ni-All	-			-		
Ls GC Feeding		Ni-Sediment	Ni-Food	Ni-All	Ni-Water	Controls	*p* -value
	Ni-Food	+		+	+	+	< 0.001
	Controls	-		-	+		
	Ni-All	-			+		

(+ Tukey's significant effect)

(- Tukey's non-significant effect)

Ls = *Lymnaea stagnalis*

Ha = *Hyalella azteca*

GC= Greenville Creek sediments

WD= Warden Ditch sediments

Table 6-4. *Lymnaea stagnalis* weights for all treatments during the 7 d Ni sediment tests.

			Treatments							
Date	**Exposure compartment**		**WD Ref**	**WD TSS**	**WD DOC**	**WD Ni-only**	**GC Ref**	**GC TSS**	**GC DOC**	**GC Ni-only**
8-Apr-10	Ni-water	Mean (mg dry wt)	2.13	1.37	1.67	0.93	1.70	0.97	1.37	1.00
		St. Dev (mg dry wt)	0.12	0.15	0.15	0.45	0.26	0.29	0.15	0.10
7-May-10	Ni-sediment	Mean (mg dry wt)	2.00	2.57	1.73	1.87	1.93	0.00	0.00	0.00
		St. Dev (mg dry wt)	0.17	0.93	0.06	0.32	0.32	0.00	0.00	0.00
27-May-10	Ni-food	Mean (mg dry wt)	2.67	1.93	1.73	1.70	2.57	1.97	2.20	1.87
		St. Dev (mg dry wt)	0.31	0.40	0.15	0.30	0.32	0.35	0.57	0.81
16-Jun-10	Ni-all compartments	Mean (mg dry wt)	1.10	0.73	0.90	0.77	1.17	0.83	1.10	0.43
		St. Dev (mg dry wt)	0.10	0.32	0.17	0.23	0.32	0.15	0.17	0.06
30-Oct-10	Controls	Mean (mg dry wt)	2.83	2.60	1.93		2.27	2.07	2.33	
		St. Dev (mg dry wt)	0.25	0.44	0.23		0.21	0.32	0.42	

WD = Warden Ditch sediment

GC = Greenville Creek sediment

TSS = Total suspended solids

DOC = Dissolved organic carbon

Ni = Nickel

Table 6-5. Lettuce disc loss from *Lymnaea stagnalis* feeding during the 7 d Ni sediment tests.

			Treatments							
Date	**Exposure compartment**		**WD Ref**	**WD TSS**	**WD DOC**	**WD Ni-only**	**GC Ref**	**GC TSS**	**GC DOC**	**GC Ni-only**
8-Apr-10	Ni-water	Mean (mg wet wt)	12.70	16.90	10.87	17.10	11.90	20.20	13.17	21.10
		St. Dev (mg wet wt)	4.37	0.62	0.72	2.60	4.78	4.91	8.80	5.67
7-May-10	Ni-sediment	Mean (mg wet wt)	12.60	-8.57	28.43	22.20	10.47	24.60	35.43	10.77
		St. Dev (mg wet wt)	9.75	26.61	11.55	9.95	22.93	7.87	13.80	8.06
27-May-10	Ni-food	Mean (mg wet wt)	-20.30	-20.67	-37.83	-28.57	-31.23	-32.83	-28.00	-47.10
		St. Dev (mg wet wt)	18.60	11.83	21.75	22.22	17.86	13.50	27.01	27.12
16-Jun-10	Ni-all compartments	Mean (mg wet wt)	-6.70	-2.37	-10.83	-4.83	-13.13	-4.77	-4.83	1.50
		St. Dev (mg wet wt)	4.49	1.91	1.10	3.85	11.36	4.01	4.28	4.67
30-Oct-10	Controls	Mean (mg wet wt)	-12.77	-3.37	-6.63		-3.30	-7.63	-7.23	
		St. Dev (mg wet wt)	6.71	4.51	3.20		4.45	1.59	2.44	

WD = Warden Ditch sediment

GC = Greenville Creek sediment

TSS = Total suspended solids

DOC = Dissolved organic carbon

Ni = Nickel

Table 6-6. *Lymnaea stagnalis* ^{62}Ni whole body burden concentrations, and ^{62}Ni food, water, and Trophic Transfer Factor ratios for the Ni-Food experiment.

Nickel spike	Treatment	^{62}Ni in Organism (mean) (µg/g)	^{62}Ni in Organism (St.dev) (µg/g)	Statistical result	^{62}Ni in Food (mean) (µg/g)	^{62}Ni in Food (St.dev) (µg/g)	^{62}Ni org/total Ni food (ng/g) Mean	^{62}Ni org/total Ni food (ng/g) St.dev	Statistical result	^{62}Ni org/total Ni water Mean (µg/l)	^{62}Ni org/total Ni water St.dev (µg/l)	Statistical result	TTF (ng/g) ^{62}Ni Mean	TTF (ng/g) ^{62}Ni St.dev	Statistical result
Food *Lymnaea stagnalis*	WD Ref Ls	0.9	0.7	p = 0.109	0.5	0.6	0.04	0.04	p = 0.579	2830	2125	a p = 0.034	0.53	0.66	p = 0.516
	WD TSS-Ni Ls	7.6	4.0		104.3	23.4	0.08	0.05		18051	9437	ab	0.08	0.06	
	WD DOC-Ni Ls	8.0	4.9		48.3	36.7	0.08	0.06		16321	10086	c	0.19	0.10	
	WD None Ls	6.5	2.5		53.0	78.0	0.03	0.04		5389	2089	d	0.16	0.25	
	GC Ref Ls	0.5	0.3	a p < 0.001	4.7	3.6	0.01	0.01	a p = 0.025	1462	772	a p = 0.034	0.20	0.24	p = 0.595
	GC TSS-Ni Ls	13.0	3.2	ab, cb	84.4	39.1	0.03	0.03	b	17586	4335	ab, bc	0.19	0.14	
	GC DOC-Ni Ls	2.1	1.8	c	6.1	10.5	0.00	0.00	cd	2661	2360	c	0.06	0.11	
	GC None Ls	13.5	3.9	ad, cd	70.3	15.8	0.07	0.04	ad	15151	4370	ad, cd	0.19	0.01	

Ls = Lymnaea stagnalis
GC= Greenville Creek sediments
WD= Warden Ditch sediments
Ref = Reference
DOC = Dissolved organic carbon
TSS = Total Suspended Solids
TTF = Trophic Transfer Factor

Table 6-7. *Hyalella azteca* weights for all treatments during the 7 d Ni sediment tests.

			Treatments							
Date	**Exposure compartment**		**WD Ref**	**WD TSS**	**WD DOC**	**WD Ni-only**	**GC Ref**	**GC TSS**	**GC DOC**	**GC Ni-only**
16-Apr-10	Ni-water	Mean (mg dry wt)	0.73	0.53	0.37	0.10	0.97	0.43	0.77	0.40
		St. Dev (mg dry wt)	0.06	0.21	0.12	0.10	0.38	0.15	0.25	0.10
18-May-10	Ni-sediment	Mean (mg dry wt)	0.87	0.17	0.70	0.40	0.83	0.20	0.10	0.10
		St. Dev (mg dry wt)	0.25	0.15	0.20	0.17	0.42	0.17	0.10	0.17
7-Jun-10	Ni-food	Mean (mg dry wt)	0.57	0.50	0.80	0.63	0.63	0.70	0.43	0.63
		St. Dev (mg dry wt)	0.12	0.10	0.00	0.15	0.06	0.30	0.15	0.32
25-Jun-10	Ni-all compartments	Mean (mg dry wt)	0.80	0.57	0.83	0.67	0.93	0.47	0.43	0.17
		St. Dev (mg dry wt)	0.10	0.31	0.12	0.15	0.06	0.38	0.21	0.29
25-Oct-10	Controls	Mean (mg dry wt)	2.20	1.73	1.83		1.67	1.50	1.63	
		St. Dev (mg dry wt)	0.40	0.38	0.31		0.31	0.30	0.45	

WD = Warden Ditch sediment

GC = Greenville Creek sediment

TSS = Total suspended solids

DOC = Dissolved organic carbon

Ni = Nickel

Table 6-8. Leaf disc loss from *Hyalella azteca* feeding during the 7 d Ni sediment tests.

			Treatments							
Date	**Exposure compartment**		**WD Ref**	**WD TSS**	**WD DOC**	**WD Ni-only**	**GC Ref**	**GC TSS**	**GC DOC**	**GC Ni-only**
16-Apr-10	Ni-water	Mean (mg dry wt)	-0.77	-0.07	0.13	0.10	-0.33	0.03	-0.17	-0.33
		St. Dev (mg dry wt)	0.12	0.38	0.12	0.26	0.21	0.06	0.12	0.40
18-May-10	Ni-sediment	Mean (mg dry wt)	-1.57	-1.63	-1.83	-1.83	-0.80	-0.60	-0.37	-0.57
		St. Dev (mg dry wt)	0.84	1.07	0.45	0.31	0.00	0.75	0.38	0.35
7-Jun-10	Ni-food	Mean (mg dry wt)	-0.97	-0.93	-0.83	-0.70	-0.40	-0.27	-0.43	-0.07
		St. Dev (mg dry wt)	0.49	0.23	0.38	0.53	0.46	0.12	0.06	0.12
25-Jun-10	Ni-all compartments	Mean (mg dry wt)	-1.33	-0.10	0.13	-0.10	-0.57	-0.03	-0.07	0.47
		St. Dev (mg dry wt)	0.25	0.36	0.25	0.20	0.35	0.15	0.06	0.29
25-Oct-10	Controls	Mean (mg dry wt)	-1.20	-1.67	-2.03		-0.93	-1.27	-1.10	
		St. Dev (mg dry wt)	0.20	0.21	0.40		0.42	0.59	0.30	

WD = Warden Ditch sediment

GC = Greenville Creek sediment

TSS = Total suspended solids

DOC = Dissolved organic carbon

Ni = Nickel

Table 6-9. *Hyalella azteca* ^{62}Ni whole body burden concentrations, and ^{62}Ni food, water, and Trophic Transfer Factor ratios for the Ni-Food experiment.

Nickel spike	Treatment	^{62}Ni in Organism (mean) (µg/g)	^{62}Ni in Organism (St.dev) (µg/g)	Statistical result	^{62}Ni in Food (mean) (µg/g)	^{62}Ni in Food (St.dev) (µg/g)	^{62}Ni org/total Ni food (ng/g) Mean	^{62}Ni org/total Ni food (ng/g) St.dev	Statistical result	^{62}Ni org/total Ni water Mean (µg/l)	^{62}Ni org/total Ni water St.dev (µg/l)	Statistical result	TTF (ng/g) ^{62}Ni Mean	TTF (ng/g) ^{62}Ni St.dev	Statistical result
Food *Hyalella azteca*	WD Ref Ha	1.0	0.6	a p = 0.009	0.3	0.2	0.15	0.08	a p = 0.025	2684	1431	a p = 0.001	3.26	0.71	a p = 0.034
	WD TSS-Ni Ha	9.8	4.6	ab, bc, bd	413.3	30.1	0.02	0.01	ab	39174	18174	ab	0.02	0.01	ab
	WD DOC-Ni Ha	1.9	0.7	c	339.5	37.8	0.01	0.00	ac	2924	1121	bc	0.01	0.00	ac
	WD None Ha	2.3	1.9	d	327.0	33.4	0.01	0.00	ad	3738	3026	bd	0.01	0.01	ad
	GC Ref Ha	5.5	4.2	p = 0.746	0.3	0.1	1.02	0.76	p = 0.075	18934	14694	p = 0.426	19.04	15.29	p = 0.086
	GC TSS-Ni Ha	4.6	3.7		260.1	37.6	0.01	0.01		4304	3415		0.02	0.01	
	GC DOC-Ni Ha	8.5	6.7		376.6	17.4	0.01	0.01		10251	8098		0.02	0.02	
	GC None Ha	6.2	1.9		433.3	64.2	0.01	0.00		11282	3374		0.01	0.01	

Ha = Hyalella azteca
GC= Greenville Creek sediments
WD= Warden Ditch sediments
Ref = Reference
DOC = Dissolved organic carbon
TSS = Total Suspended Solids
TTF = Trophic Transfer Factor

Table 6-10. *Lymnaea stagnalis* ^{62}Ni whole body burden concentrations, and ^{62}Ni food, water, and Trophic Transfer Factor ratios for the Ni-All experiment.

Nickel spike	Treatment	^{62}Ni in Organism (mean) (µg/g)	^{62}Ni in Organism (St.dev) (µg/g)	Statistical result	^{62}Ni in Food (mean) (µg/g)	^{62}Ni in Food (St.dev) (µg/g)	Ratio ^{62}Ni/Total Ni organism (ng/g) Mean	Ratio ^{62}Ni/Total Ni organism (ng/g) St.dev	Statistical result	^{62}Ni org/total Ni food (ng/g) Mean	^{62}Ni org/total Ni food (ng/g) St.dev	Statistical result	^{62}Ni org/total Ni water Mean (pg/L)	^{62}Ni org/total Ni water St.dev (pg/L)	Statistical result	TTF (ng/g) ^{62}Ni Mean	TTF (ng/g) ^{62}Ni St.dev	Statistical result
Water, Food, Sediment	WD Ref Ls	0.2	0.3	a p = 0.040	1.2	0.1	0.05	0.08	p = 0.076	0.02	0.02	p = 0.317	422	632	p = 0.936	0.17	0.25	p = 0.789
	WD TSS-Ni Ls	7.3	4.6	ab	101.3	45.7	0.12	0.04		0.01	0.01		145	91		0.07	0.02	
	WD DOC-Ni Ls	6.9	2.6	c	83.5	25.7	0.17	0.04		0.04	0.03		120	45		0.10	0.07	
	WD Ni-only Ls	3.8	1.2	d	75.9	1.6	0.08	0.03		0.01	0.00		71	22		0.05	0.02	
	GC Ref Ls	1.9	0.8	a* p = 0.201 p = 0.046*	0.7	0.4	0.18	0.08	p = 0.862	0.19	0.16	p = 0.198	3200	1436	a p = 0.002 (not dif from TSS)	3.72	2.15	a p =<0.002 (dif from all)
	GC TSS-Ni Ls	31.5 (16.2)	26.6 (0.7)	ab*	63.1	23.5	0.16	0.03		0.10	0.07		520	439	b	0.46	0.24	ab
	GC DOC-Ni Ls	20.8 (10.5)	18.3 (6.1)	c*	97.6	26.4	0.17	0.05		0.08	0.05		227	200	ac	0.21	0.15	ac
	GC Ni-only Ls	8.3	5.7	d*	57.1	52.7	0.14	0.07		0.02	0.02		106	73	ad	0.08	0.12	ad

Ls = Lymnaea stagnalis
GC= Greenville Creek sediments
WD= Warden Ditch sediments
Ref = Reference
DOC = Dissolved organic carbon
TSS = Total Suspended Solids
TTF = Trophic Transfer Factor
(outliers removed new value)
*new statistical result w/o outlier

Table 6-11. *Hyalella azteca* ^{62}Ni whole body burden concentrations, and ^{62}Ni food, water, and Trophic Transfer Factor ratios for the Ni-All experiment.

Nickel spike	Treatment	^{62}Ni in Organism (mean) (µg/g)	^{62}Ni in Organism (St.dev) (µg/g)	Statistical result	^{62}Ni in Food (mean) (µg/g)	^{62}Ni in Food (St.dev) (µg/g)	^{62}Ni org/total Ni food (ng/g) Mean	^{62}Ni org/total Ni food (ng/g) St.dev	Statistical result	^{62}Ni org/total Ni water Mean (µg/l)	^{62}Ni org/total Ni water St.dev (µg/l)	Statistical result	TTF (ng/g) ^{62}Ni Mean	TTF (ng/g) ^{62}Ni St.dev	Statistical result
Water, Food, Sediment *Hyalella azteca*	WD Ref Ha	2.0	1.4	p = 0.294	0.1	0.2	0.73	0.76	p = 0.082	4246	2940	a p < 0.001	33.84	26.48	a p = 0.044
	WD TSS-Ni Ha	7.9	12.0		183.9	31.2	0.00	0.01		28	42	ab	0.04	0.06	ab
	WD DOC-Ni Ha	1.7	1.9		191.1	37.2	0.00	0.00		6	7	ac	0.01	0.01	ac
	WD Ni-only Ha	4.6	1.0		189.3	16.2	0.00	0.00		14	3	ad	0.02	0.00	ad
	GC Ref Ha	1.8	1.5	p = 0.739	0.2	0.1	1.08	0.70	p = 0.070	3090	2578	a p = 0.002	6.88	2.83	p = 0.070
	GC TSS-Ni Ha	3.9	3.6		261.0	34.7	0.00	0.00		14	13	ab	0.01	0.01	
	GC DOC-Ni Ha	6.6	6.6		196.4	14.2	0.00	0.00		20	20	ac	0.03	0.03	
	GC Ni-only Ha	4.1	7.1		237.7	21.2	0.00	0.00		12	21	ad	0.02	0.03	

Ha = Hyalella azteca
GC= Greenville Creek sediments
WD= Warden Ditch sediments
Ref = Reference
DOC = Dissolved organic carbon
TSS = Total Suspended Solids
TTF = Trophic Transfer Factor

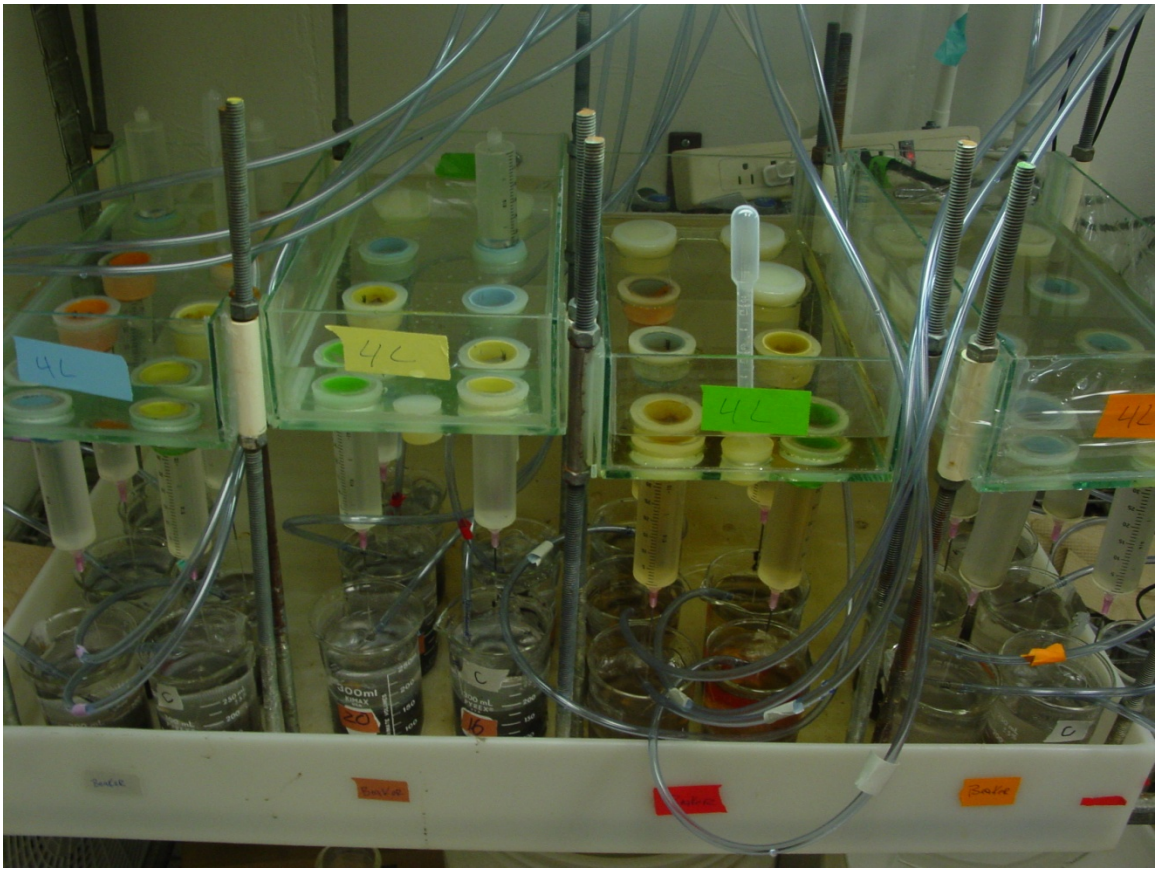

Figure 6-1. Ni tests receiving TSS, DOC, and Ni-amendments to water, sediment, and food. Airlines used to suspend TSS amendments.

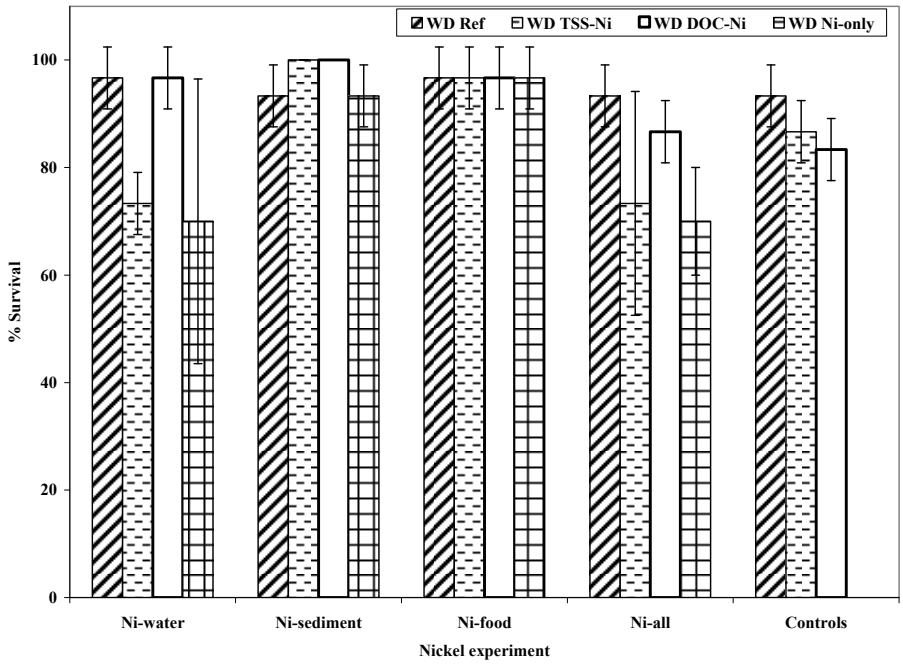

Figure 6-2. *Lymnaea stagnalis* survival on Warden Ditch sediments (WD) in all the Ni experiments.

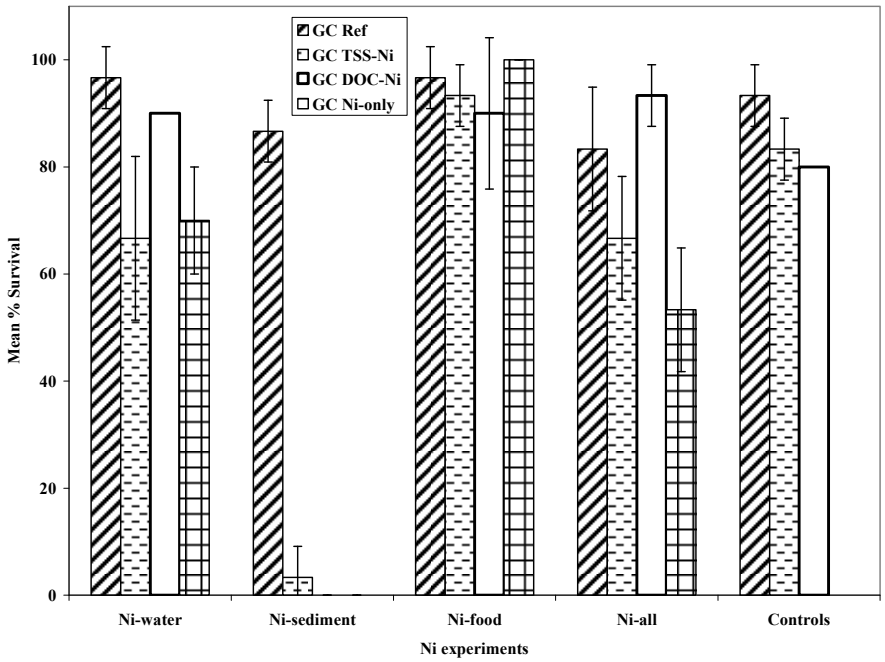

Figure 6-3. *Lymnaea stagnalis* survival on Greenville Creek sediments (GC) in all the Ni experiments.

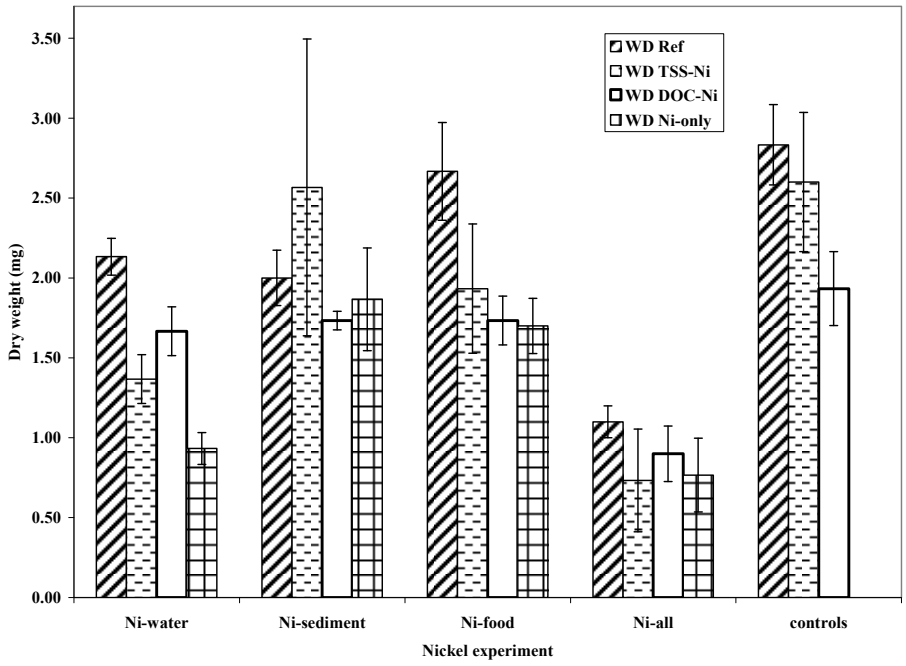

Figure 6-4. *Lymnaea stagnalis* dry weights on Warden Ditch sediments (WD) in all the Ni experiments.

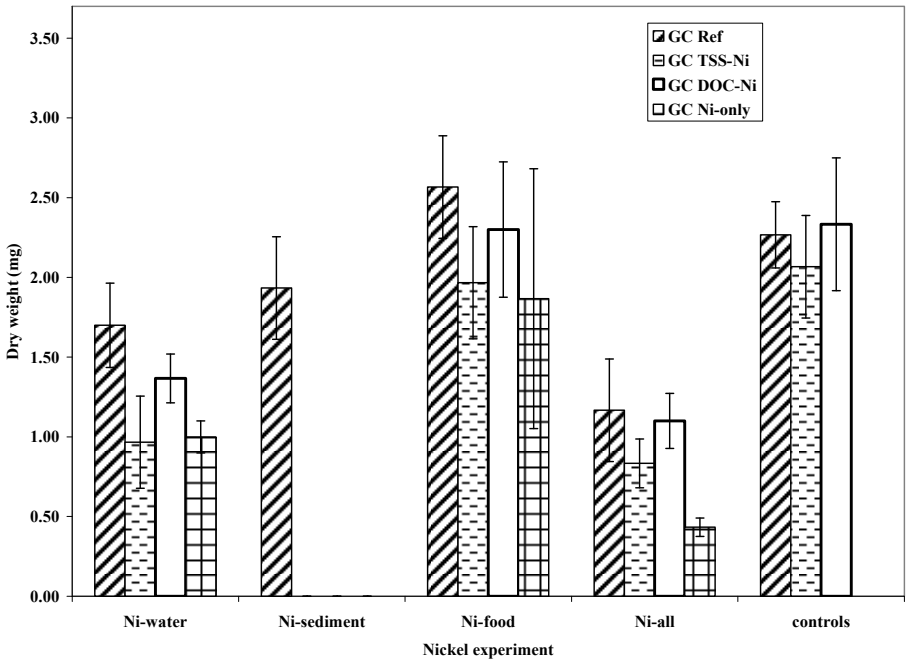

Figure 6-5. *Lymnaea stagnalis* dry weights on Greenville Creek sediments (GC) in all the Ni experiments.

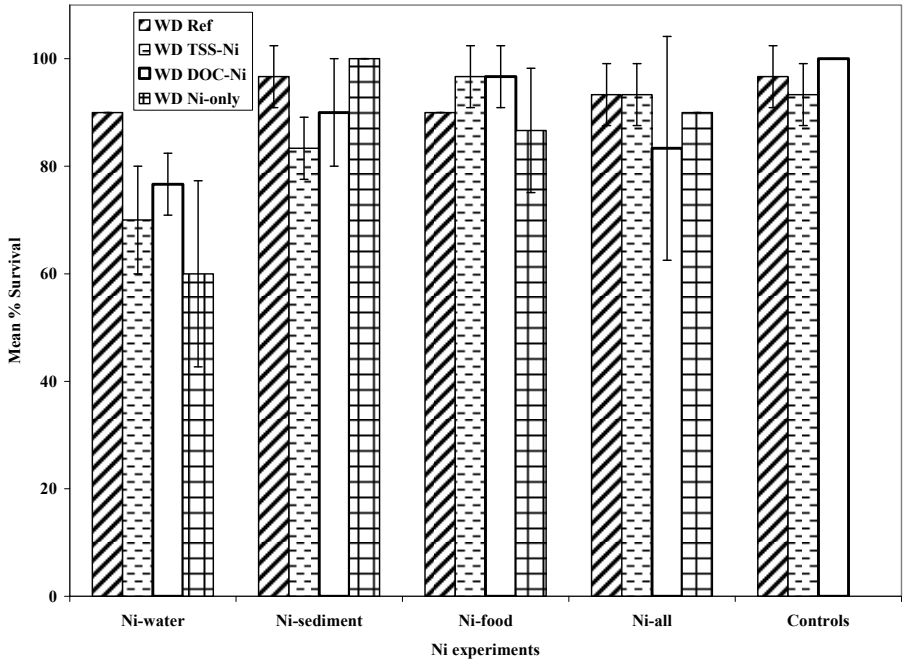

Figure 6-6. *Hyalella azteca* survival on Warden Ditch sediments (WD) in all the Ni experiments.

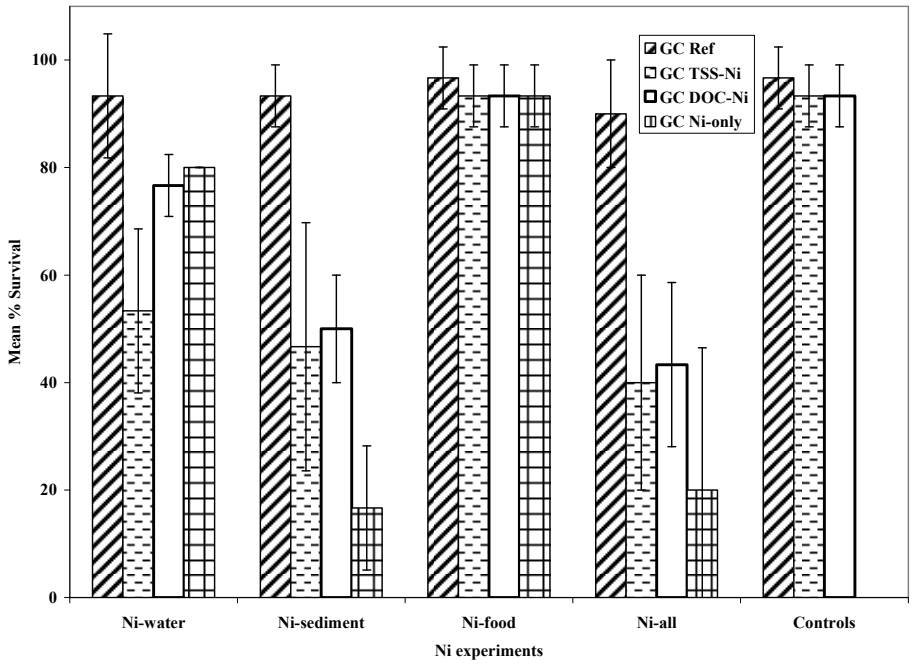

Figure 6-7. *Hyalella azteca* survival on Greenville Creek sediments (GC) in all the Ni experiments.

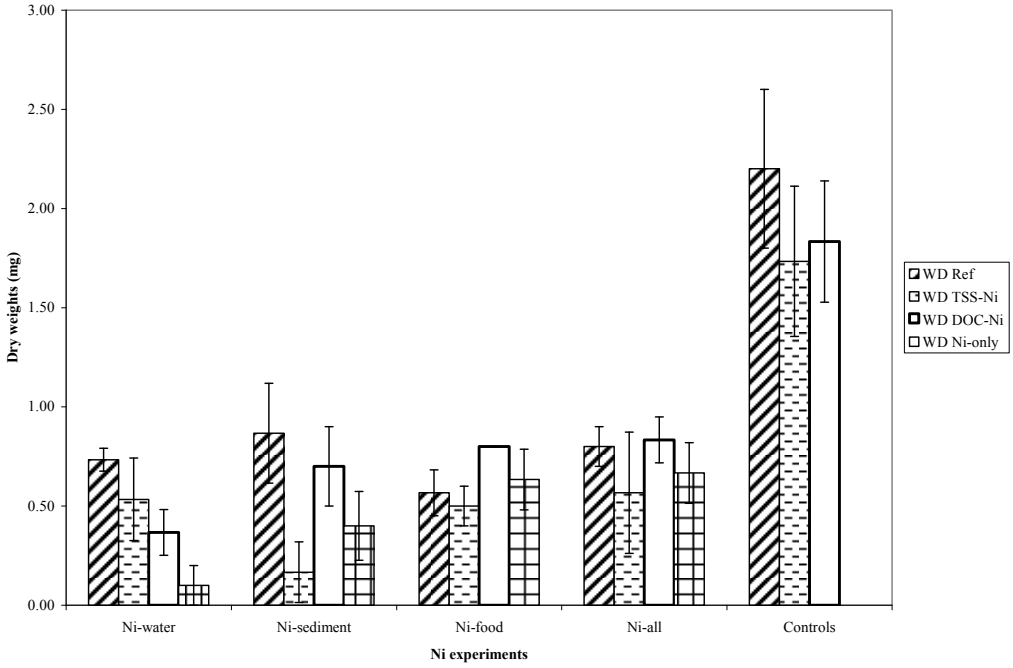

Figure 6-8. *Hyalella azteca* dry weights on Warden Ditch sediments (WD) in all the Ni experiments.

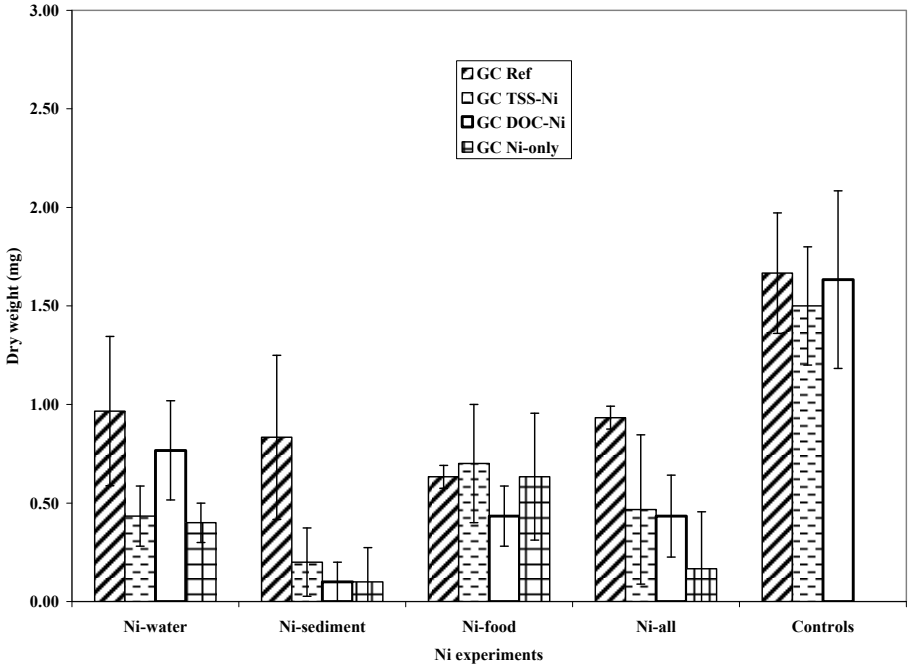

Figure 6-9. ***Hyalella azteca*** **dry weights on Greenville Creek sediments (GC) in all the Ni experiments.**

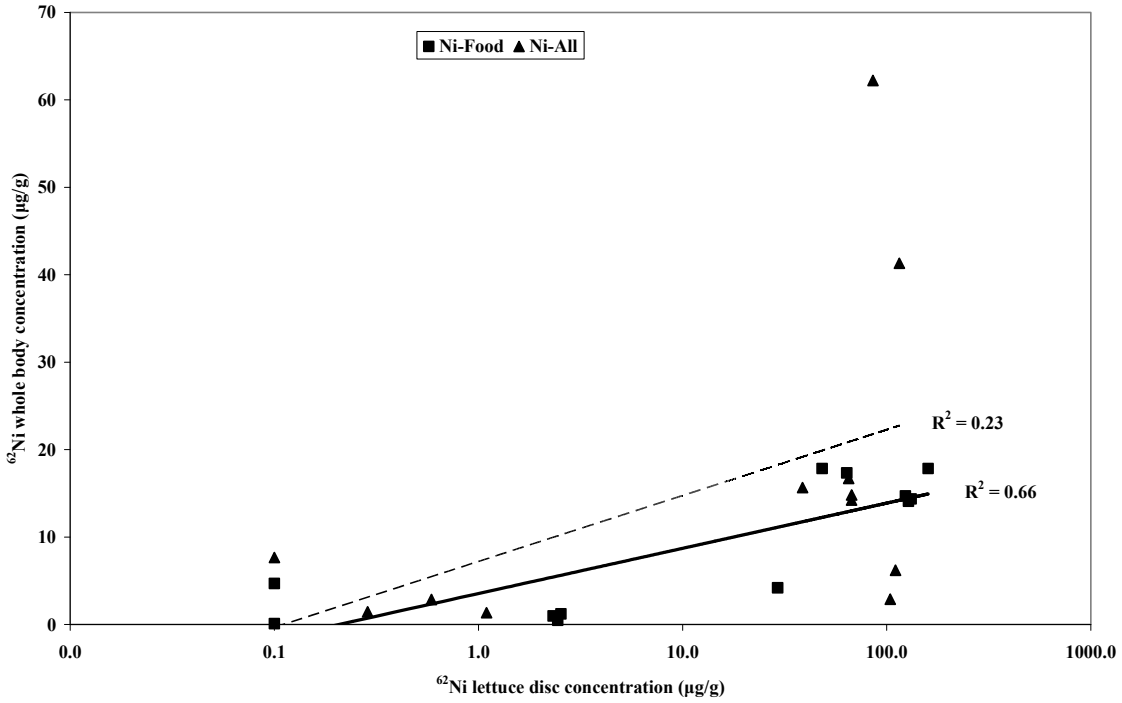

Figure 6-10. ***Lymnaea stagnalis*** **^{62}Ni whole body burden concentrations versus ^{62}Ni lettuce concentrations on GC sediments. Solid line is Ni-food test, and dashed line is Ni-all test.**

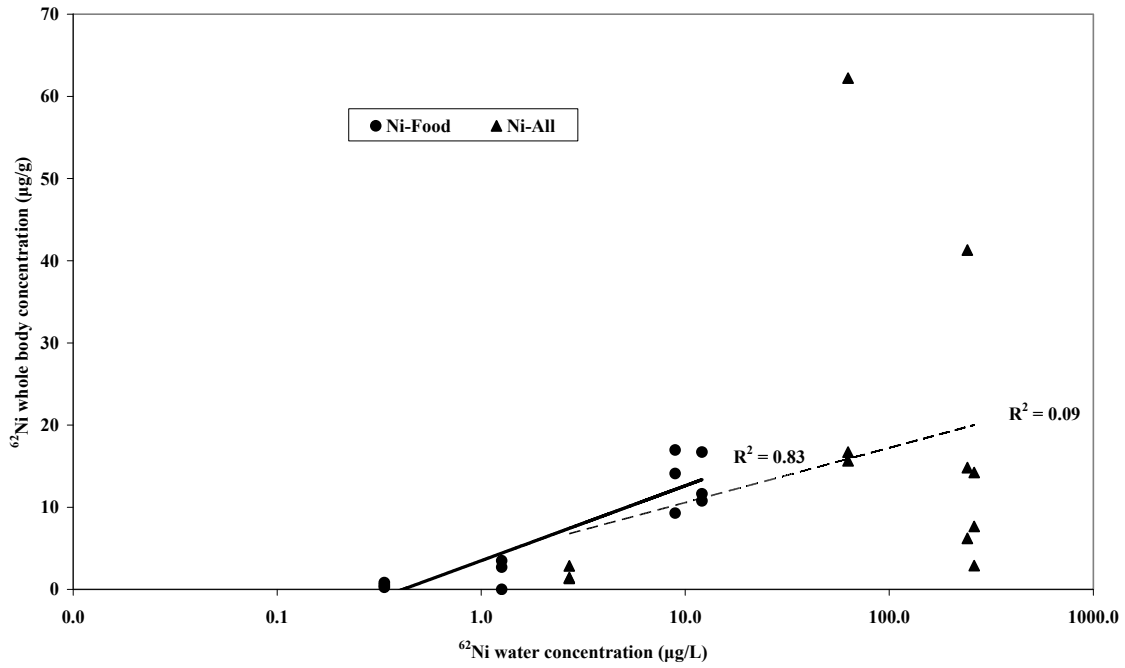

Figure 6-11. ***Lymnaea stagnalis*** **^{62}Ni whole body burden concentrations versus ^{62}Ni water concentrations on GC sediments. Solid line is Ni-food test, and dashed line is Ni-all test.**

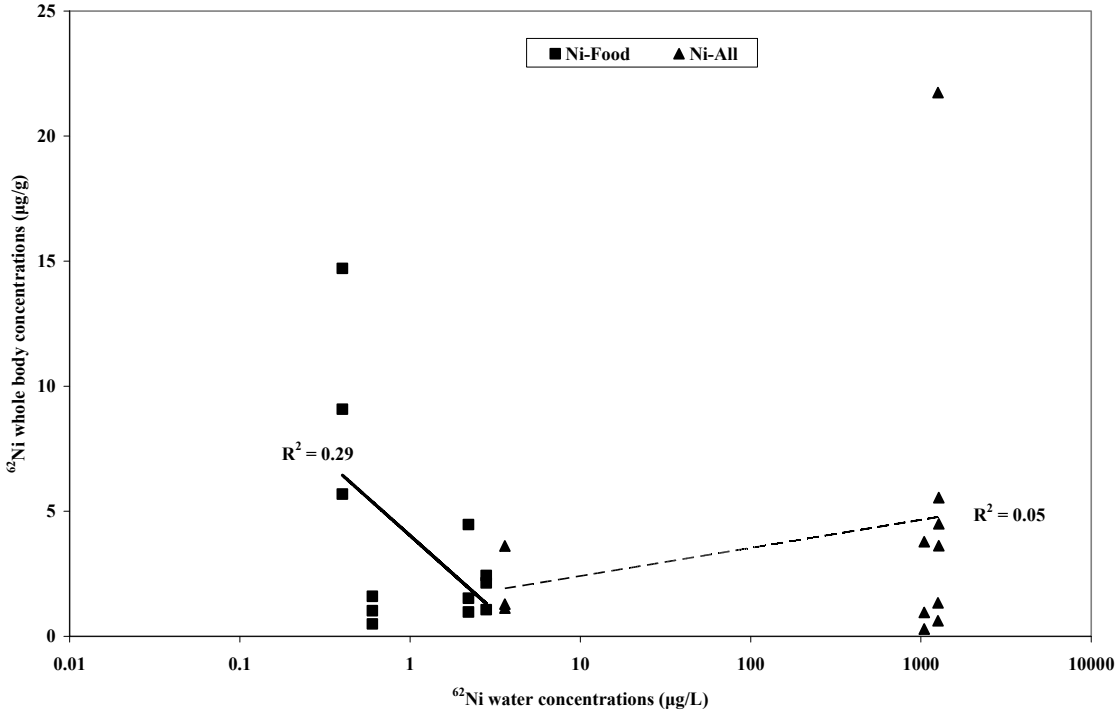

Figure 6-12. ***Hyalella azteca*** **^{62}Ni whole body burden concentrations versus ^{62}Ni water concentrations on WD sediments. Solid line is Ni-food test, and dashed line is Ni-all test.**

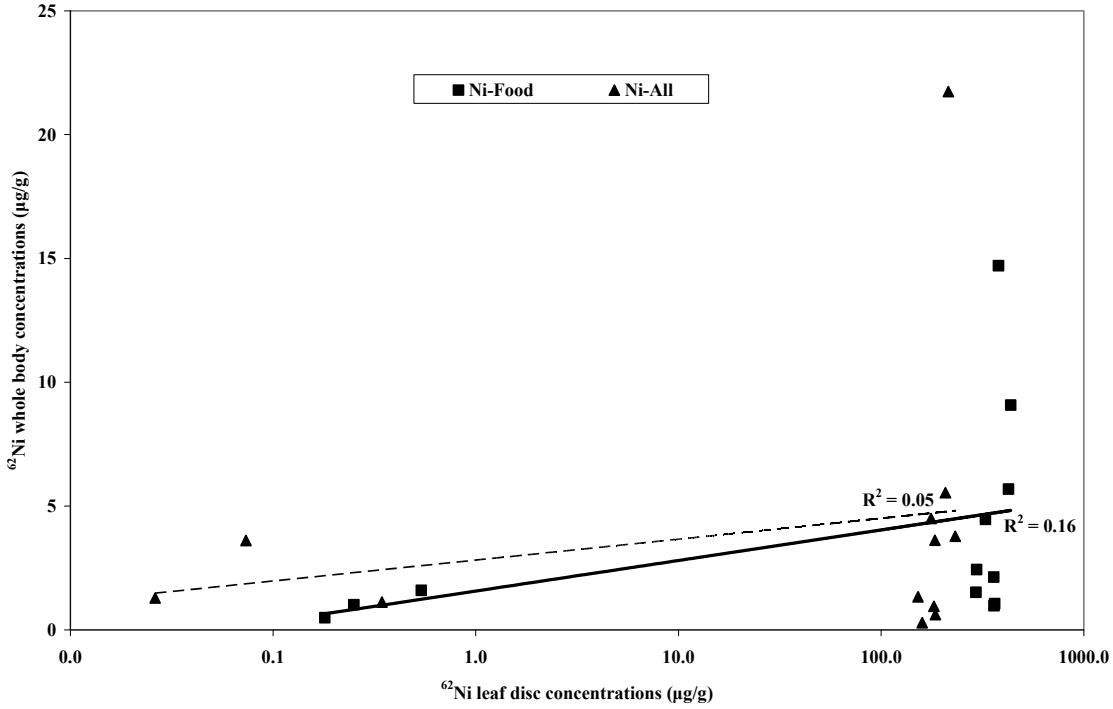

Figure 6-13. ***Hyalella azteca*** **^{62}Ni whole body burden concentrations versus ^{62}Ni leaf disc concentrations on WD sediments. Solid line is Ni-food test, and dashed line is Ni-all test.**

CHAPTER 7 – SIGNIFICANCE OF RESEARCH

In this dissertation, Ni toxicity was examined using different sediment types, systems (laboratory, mesocosms, and streams), and sites to determine if bioavailability of Ni was affected. The following is a summary of Ni effects observed on benthic communities and individual aquatic species. Ni has been an overlooked metal in the realm of aquatic ecotoxicity testing, mainly because it is less toxic than most other heavy metals (Cu, Zn, Cd, and Pb). This research has shown that Ni is toxic, and sediment characteristics (AVS, OC), substrate type (oxic vs. anoxic), site, and overlying water chemistry (DOC, hardness, TSS) all play an important role in aquatic organism toxic responses.

In Chapter 2, Ni toxicity to transplanted benthic macroinvertebrate communities were tested in a streamside mesocosm. Increased sediment porewater Ni was bioavailable to benthic organisms in both sediment types, and showed decreased macroinvertebrate abundances (Chironomidae, Hyalellidae, Crangonyctidae) on the two sediment types (low AVS and OC, and high AVS, OC). AVS and OC attenuated Ni toxicity, as shown by benthic communities responding negatively to increasing SEM_{Ni}/AVS and (SEM_{Ni}-AVS)/*foc* models. Benthic macroinvertebrate communities were demonstrating a preference for colonizing the low AVS, OC (MR) sediments, even though overall Ni bioavailability was generally higher than in the WD sediments.

In Chapter 3, Ni toxicity was examined further in a streamside mesocosm in a stream (Stillwater River) which had little to no blue green algae. Ni flux was confirmed in both sediment types, and differences in flux were observed between sediment types (GC and BC). Benthic communities (EPT taxa) were responding negatively to increasing bioavailable Ni (SEM_{Ni}/AVS models, SEM_{Ni}, and total Ni), and were similar to Chapter 2 results. The results were showing that benthic communities preferred the more oxic GC sediment over the anoxic BC sediment. Deviations in theoretical SEM_{Ni}/AVS no effect levels were observed, and are suggesting that the model is not applicable to all sediment types. The benthic community results and sediment chemistry (e.g. sediment pH, AVS, TOC, and SEM_{Ni}) can help contribute to validating SEM/AVS threshold effect level.

In Chapter 4, sediment Ni toxicity was tested on four indigenous aquatic insects (*A. verticis*, *Stenonema spp*., *Isonychia spp*., and *P. herricki*) and two surrogate organisms (*H. azteca* and *C. dilutus*) in a laboratory flow-thru design. An alternative SEM_{Ni} extraction method was tested to help extract SEM_{Ni} in sediments with low AVS content. Indigenous aquatic insect Ni effects varied during the flow-thru exposures, and the mayflies, *A. verticis* and *Stenonema spp.* were the most sensitive to Ni-spiked sediments in this study. Of all organisms tested, *H. azteca* was the most sensitive to Ni. SEM_{Ni}/AVS threshold no effect levels were calculated for GC sediments and *A. verticis* was the most sensitive of all six organisms. SEM_{Ni}/AVS deviations from theoretical models were also observed in this study. This study demonstrated that Ni threshold

effect levels were different for all species, and Ni sensitivities were established for four new species. Also, the abbreviated SEM_{Ni} extraction method appears to be acceptable for sediments with low AVS content.

In Chapter 5, Ni toxicity was determined on examining benthic macroinvertebrate community colonization on two different sediment types (GC and WD) at three different sites (GC, WD, LMR). Ni flux was observed in this study, and has been documented in all Ni studies in this dissertation (Chapters 2-4). However, Ni flux from sediments appeared to be attenuated when Ni-spiked WD sediments were placed back at the WD site. This Ni loss was likely attenuated by limiting oxidation of WD sediments when placed back in similar anoxic conditions.

A number of benthic metrics (taxa richness, abundance, and EPT taxa) were responding negatively to increasing SEM_{Ni} and SEM_{Ni}/AVS values. Site (GC, WD, and LMR) differences were observed in community responses, with the GC site having highest taxa richness and total abundance, and WD site having the lowest taxa richness and abundance. Macroinvertebrate communities responded negatively with lower hardness and higher DOC. Benthic communities preferred GC sediments over WD sediments at two of the three sites. The WD sediments at GC and LMR showed lower % EPT taxa, % Ephemeroptera, % Trichoptera, and abundance. Site comparisons are complex, and need to be considered carefully, due to diversity and abundances responding to spatial and temporal factors. Deviations to the SEM_{Ni}/AVS theoretical values were observed, and this data was consistent with the previous chapters. Benthic

macroinvertebrates exposed to Ni were having a negative effect community richness and diversity, and these results are similar to the results presented in Chapters 2-4.

In Chapter 6, *H. azteca* and *L. stagnalis* responses (survival, growth, feeding, and bioaccumulation) were tested in a variety of Ni phases and systems. Ni was amended to water, sediment, and food in singular and in combination of all three. Two different sediment types were used, and overlying water was amended with DOC and TSS.

In this study DOC did show protective Ni effects on *L. stagnalis* (growth, survival, ^{62}Ni bioaccumulation) in Ni exposures. TSS inhibited *L. stagnalis* feeding in Ni-water and Ni-sediment exposures, and provided a synergistic effect on ^{62}Ni bioaccumulation. *Lymnaea stagnalis* was more sensitive than *H. azteca*, and *L. stagnalis* did respond with the selected endpoints in all Ni-exposures. *H. azteca* growth effects occurred in all Ni-exposures, and also showed a synergistic effect with TSS concentrations (28-50 mg/L) on GC sediments. *Hyalella azteca* Ni sublethal and lethal endpoints were showing decreased effects in WD ditch sediments when compared responses on GC sediments.

The results from the ^{62}Ni-food studies have demonstrated that ^{62}Ni was accumulating in both *H. azteca* and *L. stagnalis* during multiple Ni exposures with TSS and DOC water amendments on two different sediment types. Higher ^{62}Ni water ratios are from ^{62}Ni desorbing from food to the water column, and these ^{62}Ni concentrations in the water column were controlling ^{62}Ni bioaccumulation than ^{62}Ni-food alone. Food

(diet) labeled with ^{62}Ni was contributing to any adverse survival or growth effects in *L. stagnalis* and *H. azteca*.

The results from this study have demonstrated that Ni is toxic to aquatic organisms in multiple compartment and experimental designs. Ni field effects have demonstrated the importance of natural conditions (physico-chemical parameters), and how these help bring a level of ecological relevance to the results. Benthic macroinvertebrate communities in the streamside mesocosm and during the *in situ* colonization experiments were showing benthic community structure differences in the presence of increasing Ni. The flow-thru Ni sediment tests revealed new sensitive aquatic species to Ni, and these results can be extrapolated to benthic populations and communities. Finally, showing the importance of Ni amendments to water, sediment, food, and combinations of them all, and how toxicity differed on the lethal and sublethal levels, demonstrates the need to understand the compartment mechanism and its contribution to bioavailability of Ni. All of these exposures and designs have allowed for the examination of Ni bioavailability on aquatic organism, and to the insight of potential food chain, trophic level, and ultimately ecosystem level effects. Contaminated sediments are highly important to ecosystem function, health, and integrity, and metals (Ni) can compromise all of these factors. As we advance in the science and study of contaminated sediments, a better understanding of metal bioavailability, toxicity, bioaccumulation, and sediment quality guidelines are needed to understand how science

can be used to protect the many uses which aquatic ecosystems provide to wildlife, fisheries, and humans.

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